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14. ABSTRACT

Using the TRACK-TBI (***T**ransforming **R**esearch and **CL**inical **K**nowledge in **TBI)*** dataset we have created an Information Commons that integrates clinical, imaging, proteomic, genomic, and outcome biomarkers based upon the domains of the TBI Common Data Elements. The comprehensive TBI-CDE outcome measures allow for analyses of biomarker associations with a variety of measures. Available prognostic models have been evaluated against new prognostic models for TBI and found to be unsatisfactory using a multivariate approach that goes beyond the crude definitions of Mild, Moderate and Severe TBI. The latest neuroimaging methods including Quantitative CT, DTI, and resting-state functional MRI are surpassing other methods for predicting TBI patient outcomes. In emergency settings where high resolution neuroimaging is not available, rapid measurement of proteomic markers is appearing to be a valuable adjunct to current screening practices for ruling out TBI. Most importantly improving the collection of biomarkers in TBI patients will be vital to the design of future clinical trials.

15. SUBJECT TERMS
Traumatic Brain Injury; Common Data Elements; Prognosis; Outcomes

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1. INTRODUCTION

Traumatic Brain Injury (TBI) remains one of the greatest unmet needs in military and civilian medicine. The overall goal of the study is to extensively analyze the *existing* data set from the multicenter pilot study entitled TRACK-TBI: *Transforming Research and Clinical Knowledge in TBI.* TRACK-TBI represents the largest multivariate TBI database across the injury spectrum from concussion to coma with CT/MRI imaging, blood biospecimens and outcome assessments. This DoD TRACK-TBI project is undertaking more extensive analysis of this highly granular cohort of TBI subjects. This work is vital to advancing our understanding of TBI and improving prognostic methods to identify individuals at risk for persistent cognitive and psychological heath disorders following TBI. This project achieved the following aims:

Aim 1: To develop improved prognostic, diagnostic and outcome models for TBI.
Aim 2: To identify neuroimaging biomarkers for diagnosis and prognosis in TBI.
Aim 3: To identify proteomic and genomic associations with TBI phenotypes.

2. KEYWORDS

Traumatic Brain Injury; Common Data Elements; Prognosis; Outcomes; Neuroimaging; Proteomic; Genomics; Diffusion Tensor Imaging; Functional MRI; Machine learning; Topological Data Analysis.

3. OVERALL PROJECT SUMMARY

Aim 1: *To develop improved prognostic, diagnostic and outcome models for TBI.*

Progress (please note that referenced manuscripts within summaries are provided in the Appendix):

Task 1: Cleaning baseline data.
The clinical data in the TRACK-TBI pilot dataset has been cleaned and relational inconsistencies corrected.

Task 2: Prognostic modeling.
Subtasks 1 & 3: Validation of existing prediction models on the TRACK TBI data set and use of multivariable modeling of predictors identified in the univariable analysis.

A. The Lingsma et al manuscript (2015) built a validation model for mild TBI from the 386 patients with GCS of 13-15 from TRACK-TBI. Previously developed prognostic models were shown to have poor performance on this dataset (AUC < 0.56). Multivariable analyses showed that age, baseline psychiatric conditions and lower education were the strongest predictors of lower 3- and 6-month outcome.

Subtask 2: Univariable analysis of possibly relevant predictors of outcome in mild TBI.

A. The Haarbauer-Krupa et al manuscript (2017) evaluated the incidence of, and predictors to, 6-month PTSD in acute TBI (n=586) defined by qualifying for PTSD screening criteria on the DSM-IV using the PTSD Checklist – Civilian version (PCL-C). Patients with moderate disability (GOSE 5-6) had markedly higher incidences of positive screens on the PCL-C than other recovery levels. Significant predictors of PTSD were lower years of education, baseline psychiatric disorders, lack of marriage, and TBI due to mechanism of assault.

Task 3: Cleaning of outcome data.

- Progress: Outcome data has been cleaned and relational inconsistencies corrected on schedule. This progress enabled us to move along with the other tasks within Aim 1.

Task 4: Development of a preliminary prognostic model for mild TBI.

A. The Pirracchio et al manuscript (2016) describes using machine learning approaches to predict outcome. Standard statistical practice used for determining the relative importance of competing

causes of disease typically relies on *ad hoc* methods, often byproducts of machine learning procedures (stepwise regression, random forest, etc.). We undertook a fully automated procedure for variable importance measure based on collaborative targeted maximum likelihood estimation (cTMLE). The primary outcome was a disability score (Glasgow Outcome Scale - Extended (GOSE)) collected three months post-injury. We identified clinically important predictors among a set of risk factors using a variable importance analysis based on targeted maximum likelihood estimators (TMLE) and on cTMLE. Psychiatric history and history of hepatic associated with a poorer three-month GOSE. A negative association was also found for the three-month GOSE and ANKK1 polymorphisms.

B. The Nielson et al manuscript (2017) describes the application of a machine learning tool known as topological data analysis (TDA) that enables data-driven clustering of large amounts of data from individual subjects. TDA was applied to NIH CDEs across imaging, psychological and cognitive outcomes to cluster patients (N=586) across the full spectrum of TBI severity for data-driven comparisons to the standard GOS-E outcome measure, and to test whether any blood-based biomarkers could be identified from acute time points following injury to predict outcome trajectories. In the TDA representation of patient differences, similar patients are represented as nodes and nodes that contain at least 1 patient in common are joined by an edge. What emerges is a network map that represents the full map of the TBI syndrome across all patients, considering all measures simultaneously. By then recoloring these TBI patient maps by variables of interest we can generate new hypotheses that can then be tested using traditional statistical prediction tools (e.g. general linear models). Using the TDA clustered output (**Figure 1**), we identified a group of patients with minimal brain pathology (CT-/MR-) who showed significant decreases in GOS-E scores between 3- and 6-months post injury. Isolating these patients (N=37) in the TDA network, we discovered these patients had a positive diagnosis of PTSD at 6 months, and had a significant enrichment for the heterozygous allele of the PARP1 SNP (A/T rs3219119).

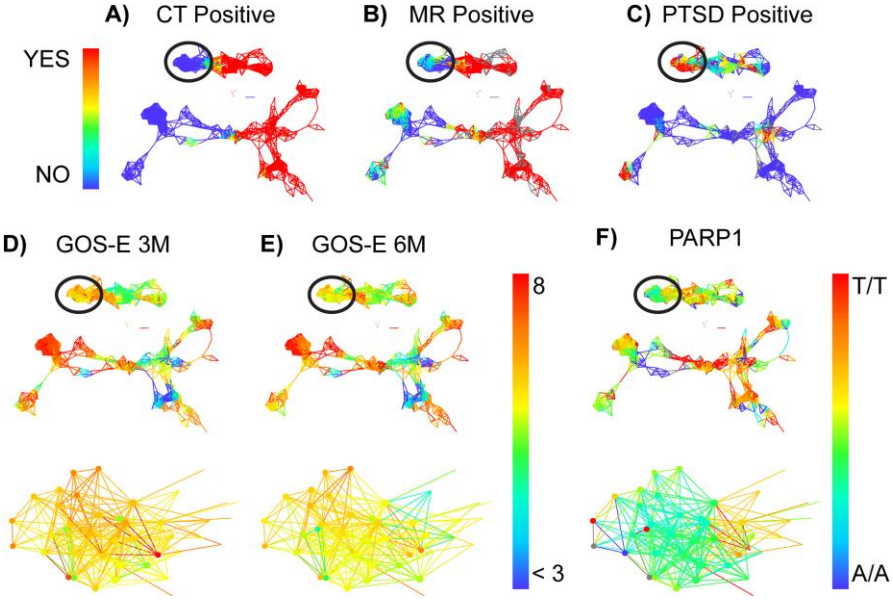

Figure 1. TDA of patients from TRACK-TBI pilot study (N=586), clustered by NIH CDEs for imaging, psychological and cognitive outcomes (Nielson et al 2017, *PLoS One*).

Furthermore, we confirmed using traditional statistical methods with general linear models that this PARP1 SNP was predictive of poor outcome on the GOS-E over time, specifically in the mTBI patients (N=122, **Figure 2A** vs. N=138, **Figure 2B**).

The results demonstrate that TDA is a useful tool for identifying specific patient groups in a large, heterogeneous TBI clinical dataset such as the TRACK-TBI pilot, in order to cluster individuals based on multivariate phenotypes across the entire syndromic outcome space. In addition, the results demonstrate that TDA can be a powerful tool to identify robust predictors of different patient group recovery trajectories for future treatment planning and clinical decision making.
In the context of the project we created an open-access, de-identified and publically available version of the TRACK-TBI pilot dataset that can be accessed through supplemental data and metadata files from the recent publication reporting these results (Nielson et al, 2017).

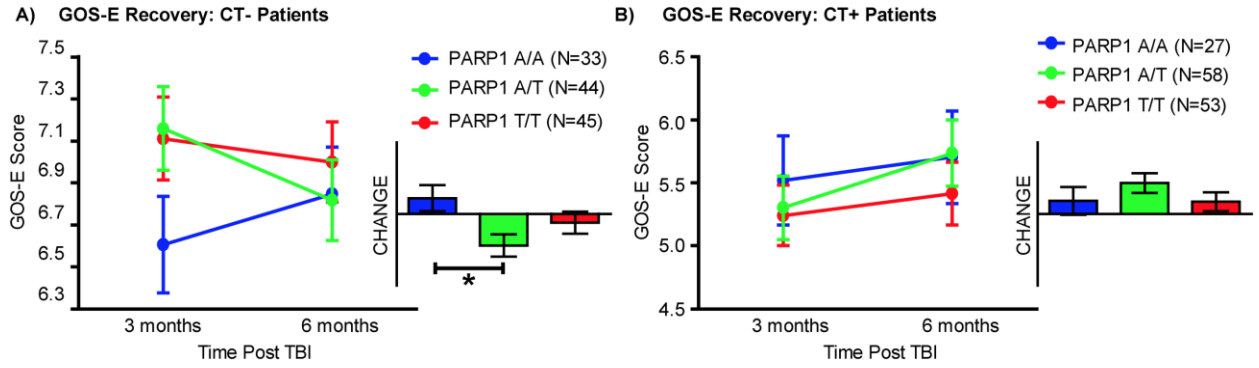

Figure 2. Traditional hypothesis testing of the predictive power of PARP1 SNP on GOS-E decline over time in mTBI patients from TRACK-TBI pilot (Nielson et al 2017, *PLoS One*). *p<.05

Table 1. Descriptive statistics of TBI patients identified for worse outcome with enrichment for PARP1 SNP, compared to all patients with the PARP1 SNP measured (Nielson et al 2017, *PLoS One*).

Patient Characteristics	All PARP1 Patients (N = 298)	TDA Subgroup (N = 37)
Age (mean & standard deviation)	43.5 +/- 18.2	41.1 +/- 14.2
Sex		
Female	91 (30.5%)	9 (24.4%)
Race		
White	252 (84.6%)	23 (62.2%)
Education		
Below high school	27 (9.1%)	9 (25%)
High school graduate	167 (56.0%)	18 (50%)
Bachelor's and above	93 (31.2%)	9 (25%)
Psychiatric History		
Present	93 (31.2%)	6 (16.2%)
Previous TBI		
No	152 (51.0%)	6 (16.2%)
Yes without hospitalization	53 (17.8%)	10 (27.0%)
Yes with hospitalization	83 (27.9%)	21 (56.8%)
Cause of Injury		
Motor vehicle accident	50 (16.8%)	3 (8.1%)
MCC/bike accident	55 (18.5%)	5 (13.5%)
Pedestrian hit	24 (6.0%)	2 (5.4%)
Fall	107 (35.9%)	11 (29.7%)
Assault	47 (15.8%)	14 (37.8%)
Other	14 (4.7%)	2 (5.4%)
ED admission GCS		
Severe (3–8)	26 (8.7%)	0 (0%)
Moderate (9–12)	13 (4.4%)	0 (0%)
Mild (13–15)	230 (77.2%)	37 (100%)
ED admission head CT		
Positive	144 (48.3%)	0 (0%)
PARP1 SNP		
A/A	67 (22.5%)	9 (37.5%)
A/T	116 (38.9%)	9 (37.5%)
T/T	115 (38.6%)	6 (25%)

Abbreviations: PARP1 = Poly [ADP-ribose] polymerase 1, TDA = topological data analysis, TBI = traumatic brain injury, MCC = motorcycle, ED = emergency department, GCS = Glasgow Coma Scale, CT = computed tomography, SNP = single nucleotide polymorphism.

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Table 1 describes the differences between descriptive characteristics of the patient group identified using TDA (N=37) to the rest of the patients with SNPs measured (N=298). There were notable differences between the data-driven group where impact of PARP1 was discovered, and the entire cohort with PARP1 measured that may be predictive of poor outcome following TBI. Regarding proportion/percentage of the respective group, the TDA group contained less females, less white patients with lower overall education levels and a much greater proportion of patients having a prior

history of TBI with hospitalization. There was, however, less history of a psychiatric illness, and most of the injuries were caused by assault, as opposed to falls in the other group.

Additional studies demonstrated the predictive power of other important SNPs in TBI patients for predicting various cognitive and psychological outcomes, including ANKK1 (Yue et al. 2015), COMT (Winkler et al. 2016; Winkler et al. 2017) and DRD2 (Yue et al. 2016). Performing traditional hypothesis testing on the predictive power of these SNPs on GOS-E outcomes over time in either CT- or CT+ TBI patients was able to demonstrate the importance of these biomarkers as potential candidates for therapeutic interventions for future patients. ANKK1 was evaluated across 3 separate variants which had different impacts on GOS-E recovery over time (**Figure 3**), revealing a significant 3-way interaction for ANKK1 Gly422Arg (rs4938016) only, and a significant difference in GOS-E scores at both 3 and 6 months for patients with a positive head CT for ANKK1 Gly318Arg (rs11604671). However, these differences were not found to significantly change over time.

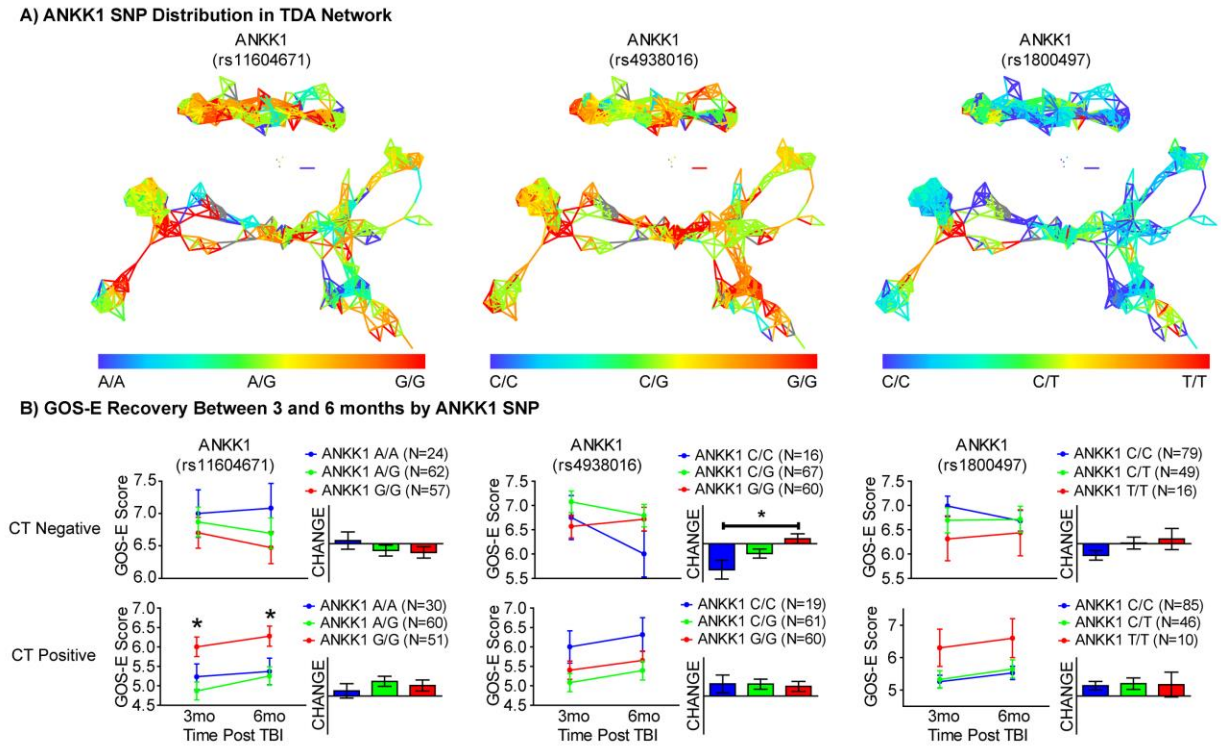

Figure 3. ANKK1 SNP distribution in TDA network and hypothesis testing on GOS-E recovery between 3 and 6 months post-TBI. *p<.05

Hypothesis testing of the interaction between CT pathology and the COMT SNP allele on GOS-E outcome over time (**Figure 4**) revealed both a significant association of COMT with GOS-E recovery over time, and a 3-way interaction of GOS-E recovery with the SNP allele and presence/absence of CT pathology, specifically in patients with negative head CT.

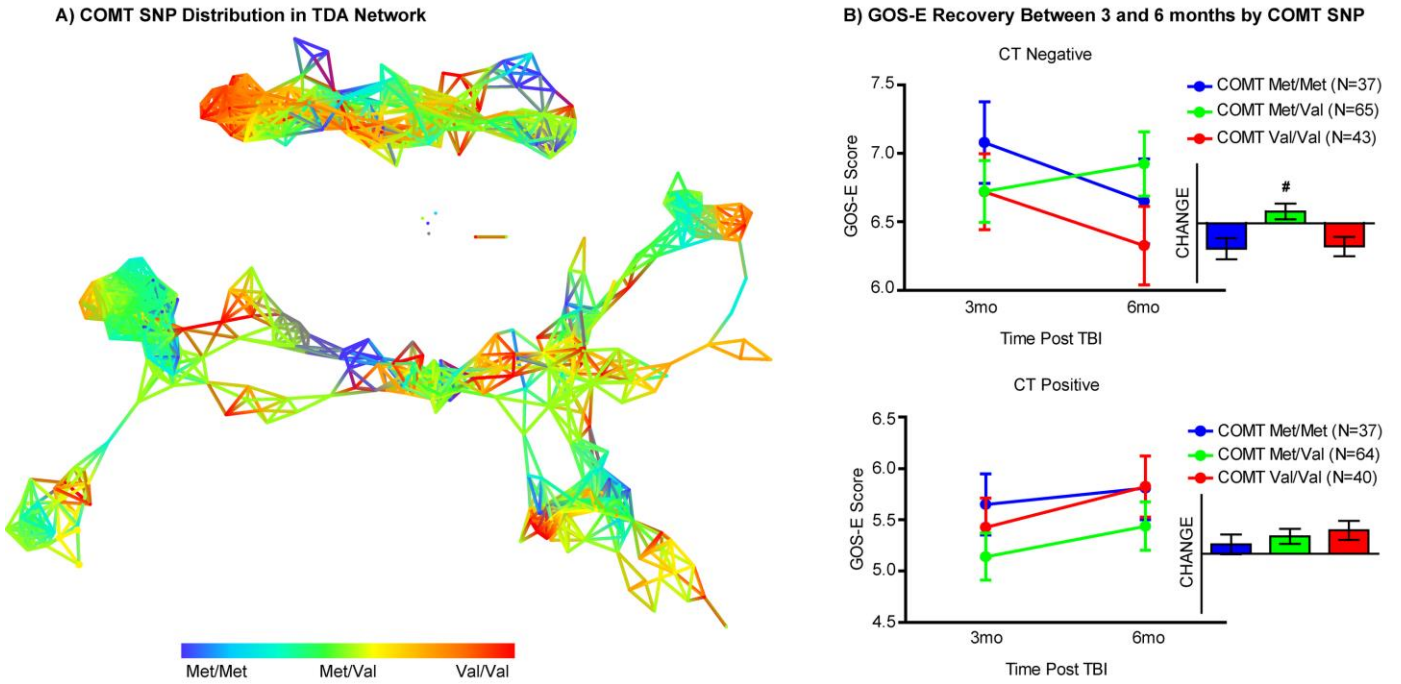

Figure 4. COMT SNP distribution in TDA network and hypothesis testing for GOS-E recovery between 2 and 6 months post-TBI. # $p < .05$ compared to both groups.

Hypothesis testing of the interaction between CT pathology and the DRD2 SNP allele on GOS-E recovery (**Figure 5**) revealed a significant association of DRD2 with GOS-E at 3 and 6 months post TBI, however this was only detected in patients with a positive head CT and did not significantly change over time

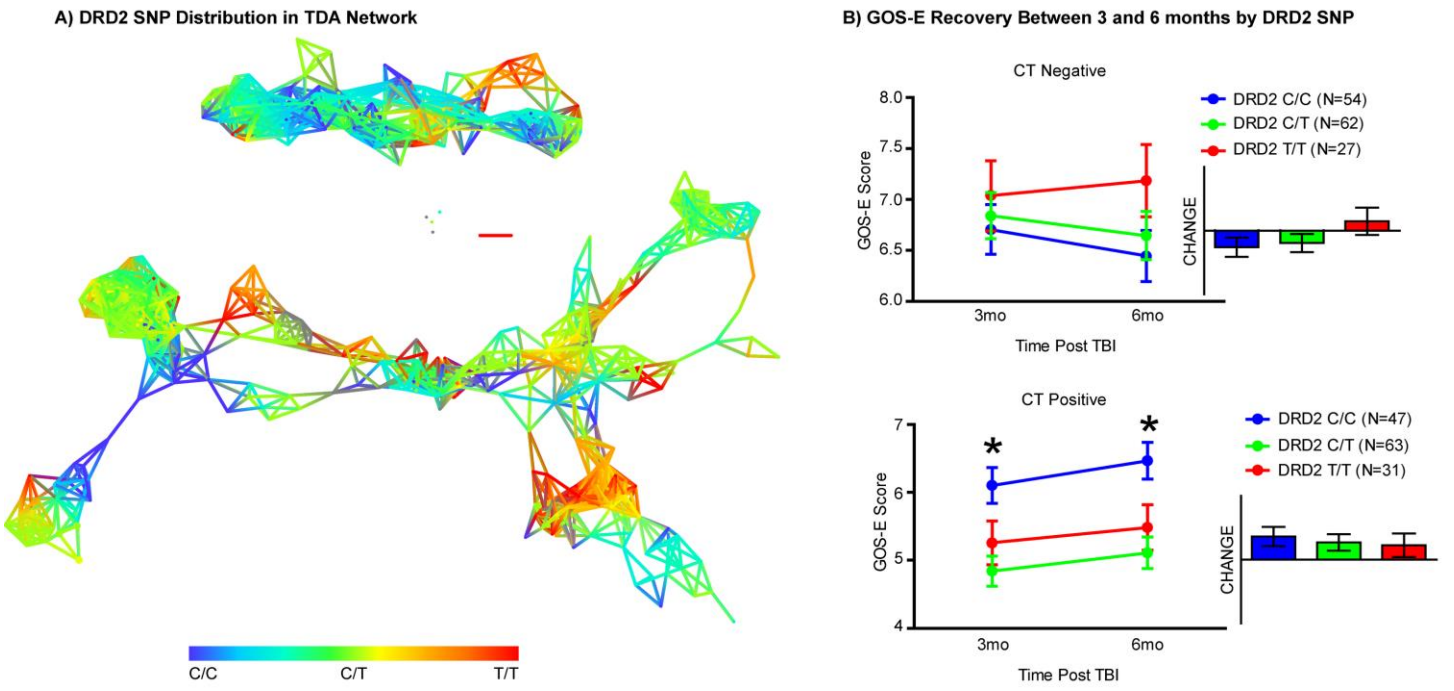

Figure 5. DRD2 SNP distribution in TDA network and hypothesis testing for GOS-E recovery between 2 and 6 post-TBI. *p < .05

Task 5. Diagnostic modeling.
The progress for this collective task is reflected in the data analysis presented within tasks of Aims 2 and 3.

Aim 2: *Neuroimaging biomarkers for diagnosis and prognosis in TBI*

Task 1. Extract imaging common data elements (CDE) from CT and MRI exams.

Progress: All CT and MRI exams have been interpreted by a board-certified neuroradiologist, and pathoanatomic lesions have been recorded using the NIH Common Data Elements (CDEs). The CT CDEs for all TRACK mTBI patients were included in the data analyzed for Ferguson et al.

Task 2. Quantitative CT.

Progress: Software written for analysis of head CT was used to analyze 50 TRACK head CT exams (*figure below at left* demonstrating epidural hematoma in blue, subarachnoid hemorrhage in red, midline falx plane as green line, and severely effaced basal cisterns as green dots). We thereby demonstrated successful application of the software, which was originally "trained" on CT exams from single row-detector CT, to exams obtained by 64 row-detector CT. We now expect the software to be generally applicable to all multislice CT scanners, including the most current 256+ detector-row models.

The software interpretations on 50 TRACK head CT exams were compared to the consensus interpretations of a board-certified neuroradiologist and a neurosurgeon. Presence or absence of midline shift was identified correctly in 47 of 50 (94%) cases, and presence or absence of cistern

effacement identified correctly in 48 of 50 (96%) of cases when compared to the consensus interpretation.

Task 3. Diffusion tensor imaging (DTI) and resting-state fMRI preprocessing.
Progress: Brain extraction, eddy-current correction and motion correction, and extraction of DTI parameters using the fMRIB software library have been performed on all TRACK-TBI brain MRI exams. The preprocessed data were used in DTI analyses described in Tasks 4 and 5 below.

Task 4. DTI and resting-state fMRI analysis.

A. The Palacios et al manuscript (2017) investigated resting-state functional MRI (rsfMRI) to assess semi-acute alterations in brain connectivity and its relationship with outcome measures assessed 6 months after injury. We compared the functional connectivity of the resting-state networks (RSNs) between patients and controls, as well as group differences in the interactions between RSNs, and related both to cognitive and behavioral performance at 6 months post-injury. Alterations were found in the spatial maps of the RSNs between mTBI patients and healthy controls in networks involved in behavioral and cognition processes **(Figure 1)**. These alterations were predictive of mTBI patients' outcomes at 6 months post-injury. Moreover, different patterns of reduced network interactions were found between the CT/MRI positive and CT/MRI negative patients and the control group. These rsfMRI results demonstrate that even mTBI patients not showing brain lesions on conventional CT/MRI scans can have alterations of functional connectivity at the semi-acute stage that help explain their outcomes. These results suggest rsfMRI as a sensitive biomarker both for early diagnosis and for prediction of the cognitive and behavioral performance of these patients.

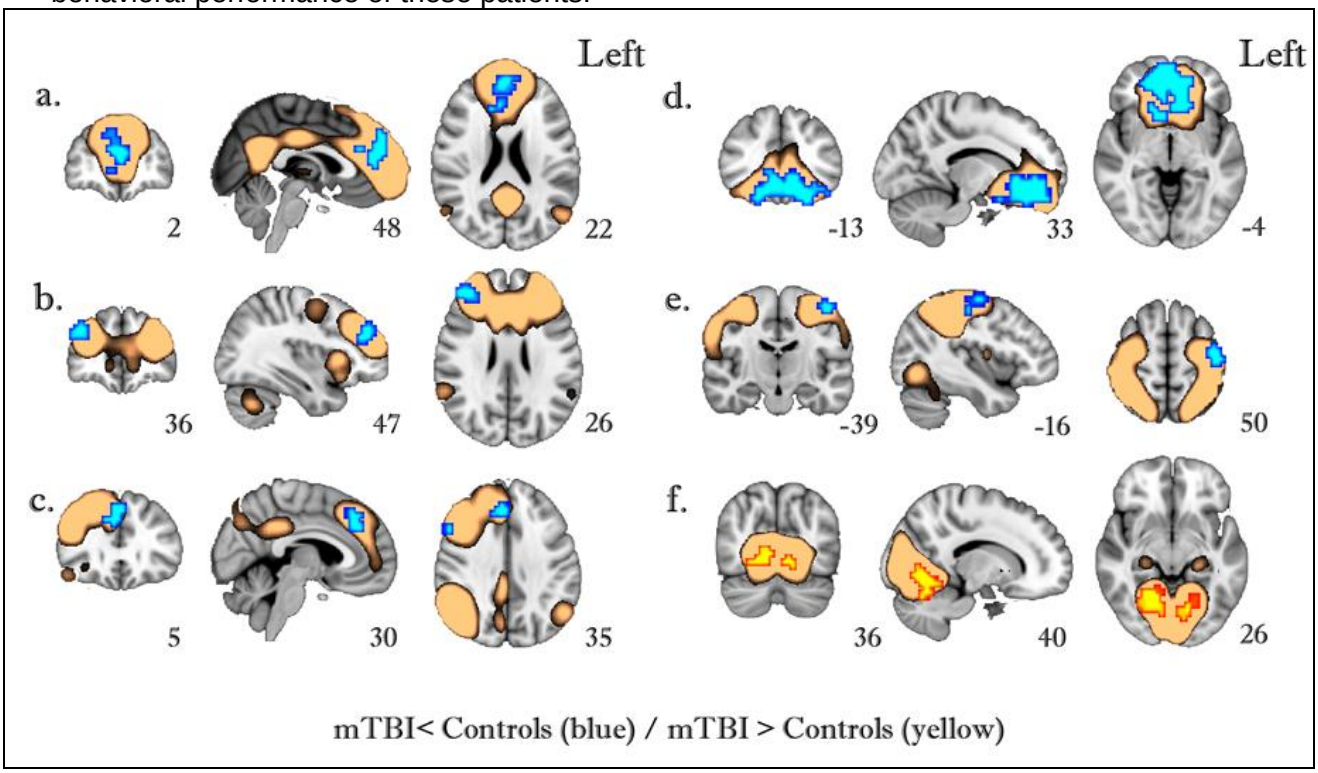

FIGURE 1: RSNs significant differences between the whole sample of mTBI patients and healthy controls. (a) DMN; (b) executive control network; (c) fronto-parietal network; (d) orbitofrontal network; (e) dorsal attentional network; (f) visual network. In blue: reductions in connectivity. In red-yellow: increases in connectivity.

Task 5. DTI and fMRI region-of-interest analysis.

A. The Yuh et al manuscript (2014) evaluated 3T diffusion tensor imaging (DTI) for white matter injury in mild traumatic brain injury (mTBI) patients employing both whole-brain voxel-wise and region-of-interest (ROI) approaches. The subgroup of 32 patients with any traumatic intracranial lesion on either day-of-injury computed tomography (CT) or semi-acute magnetic resonance imaging (MRI) demonstrated reduced fractional anisotropy (FA) in numerous white matter tracts, compared to 50 control subjects. In contrast, CT/MRI-negative mTBI patients demonstrated no significant difference in any DTI parameter, compared to controls. To determine the clinical relevance of DTI, we evaluated correlations between 3- and 6-month outcome and imaging, demographic/socioeconomic, and clinical predictors. Statistically significant univariable predictors of 3-month Glasgow Outcome Scale-Extended (GOS-E) included MRI evidence for contusion. ROI with severely reduced FA, neuropsychiatric history, age, and years of education. Significant predictors of 6-month GOS-E included ROI with severely reduced FA, neuropsychiatric history, and years of education. For the subset of patients lacking neuropsychiatric and substance abuse history, MRI surpassed all other predictors for both 3- and 6-month outcome prediction. This is the first study to compare DTI in individual mTBI patients to conventional imaging, clinical, and demographic/socioeconomic characteristics for outcome prediction. DTI demonstrated utility in an inclusive group of patients with heterogeneous backgrounds, as well as in a subset of patients without neuropsychiatric or substance abuse history.

Aim 3. To identify proteomic and genomic associations with TBI phenotypes.

Task 1. Biospecimen Proteomic Analyses.

A. The McMahon et al manuscript (2015) illustrates the novel diagnostic and prognostic utility of GFAP with potential to reduce unnecessary CT scans in emergency settings as a biomarker of brain injury. In a broad range of patients with mild to severe TBI measurement of GFAP-BDP yielded a net benefit above clinical screening alone and a net reduction in unnecessary scans by 12 to 30%. Used in conjunction with other clinical information, rapid measurement of GFAP-BDP is useful in establishing or excluding the diagnosis of radiographically apparent intracranial injury throughout the spectrum of TBI. As an adjunct to current screening practices, GFAP-BDP may help avoid unnecessary CT scans without sacrificing sensitivity.

Task 2. Biospecimen Genomic Analyses.

We validated the genetic stability of all 419 TRACK-TBI patients with DNA specimens. We have sequenced single nucleotide polymorphisms (SNPs) in 5 genes with known or high likelihood of association to TBI (ANKK1, COMT, APOE, OPRM, GABRA). The following SNPs were sequenced.

Dopaminergic:

- Rs11604671 and rs4938016 are SNPs within the DRD2 gene that form a haploblock with the already sequenced ANKK1 rs1800497 and associate with poor cognitive outcome after TBI.
- Rs6277 is a SNP within the DRD2 gene where the C allele associates with increased PTSD risk.

Serotonergic:

- Rs6311 is a SNP within the HTR2A gene in the serotonergic pathway that possibly associates with PTSD after trauma [PMID 19842167].
- Rs4795541 is a SNP within the SLC6A4 gene that associates with depression and PTSD.

Neurodegenerative:

- Rs6265 is a SNP within the BDNF gene. The A allele confers increased risk to motor skill impairment [PMID 19745020] and introversion.

Additional SNPs based on current literature: BCL2

- Rs17759659 is a SNP within the BCL2 gene which encodes a pro-survival protein in the apoptosis pathway. The SNP associates with poorer outcomes and higher mortality by GOS after severe TBI.

PARP-1

- Rs3219119 is a SNP within Poly(ADP-ribose) polymerase-1 (PARP-1) which plays an important role in cellular response to DNA damage. The AA genotype has been found to associate with favorable neurologic outcome by 6-month GOS after TBI.

Task 3. Data Analysis.
Progress: Please refer to manuscripts provided in Appendix.

Tasks 4 and 5. Prognostic and Diagnostic Modeling.
Progress: We have analyzed the allelic variation of these genes to the deeply phenotyped data from the other TRACK-TBI domains: baseline demographics, clinical course, neuroimaging on CT and MRI, and outcomes and accordingly these data are presented under Aims 1 and 3.

A. The Yue et al manuscript (2015) applied ensemble machine learning approaches to multi-analyte assays of TRACK-TBI pilot samples. This study demonstrated that the genetic biomarker ANKK1 predicts 6 month verbal outcome after TBI.

B. The Winkler et al manuscript (2015) investigated investigate whether the COMT Val158Met polymorphism influences outcome on a cognitive battery 6 months following mTBI—Wechsler Adult Intelligence Test Processing Speed Index Composite Score (WAIS-PSI), Trail Making Test (TMT) Trail B minus Trail A time, and California Verbal Learning Test, Second Edition Trial 1–5 Standard Score (CVLT-II). COMT Met158 allele associates with higher nonverbal processing speed on the WAIS-PSI when compared to Val158/Val158 homozygotes after controlling for demographics and injury severity. The COMT Val158Met polymorphism did not associate with mental flexibility on the TMT or with verbal learning on the CVLT-II. Hence, COMT Val158Met may preferentially modulate nonverbal cognition following uncomplicated TBI.

C. The Winkler et al manuscript (2017) investigated whether the COMT Val158Met polymorphism is associated with PTSD and global functional outcome as measured by the PTSD Checklist – Civilian Version and Glasgow Outcome Scale Extended (GOSE), respectively. In these mTBI subjects it was shown that the COMT Met158 allele is associated with lower incidence of PTSD and higher GOSE scores 6-months following injury. The COMT Val158Met genotype and PTSD association persists after controlling for race and pre-existing psychiatric disorders/substance abuse. PTSD emerged as a strong predictor of poorer outcome on GOSE, which persists after controlling for age, GCS, and race. When accounting for PTSD in multivariable analysis, the association of COMT genotype and GOSE did not remain significant. Whether COMT genotype indirectly influences global functional outcome through PTSD remains to be determined and larger studies in more diverse populations.

4. KEY RESEARCH ACCOMPLISHMENTS

- Publication of the first study to compare DTI in individual mild TBI patients to conventional imaging, clinical, and demographic/socioeconomic characteristics for outcome prediction. DTI demonstrated utility in an inclusive group of patients with heterogeneous backgrounds, as well as in a subset of patients without neuropsychiatric or substance abuse history. Significant predictors of 6-month GOS-E included region of interest with severely reduced fractional anisotropy, neuropsychiatric history, and years of education. For the subset of 37 patients lacking neuropsychiatric and substance abuse history, MRI surpassed all other predictors for both 3- and 6-month outcome prediction.
- Region-of-interest (ROI) analysis of fMRI functional connectivity networks were used to compare mild TBI patient s versus matched controls as well as to correlate functional connectivity at the semi-acute stage of injury to 6-month outcomes, including attentional

function and executive function with the Trail Making Test (TMT), as well as symptomatic outcome with the Rivermead Postconcussive Questionnaire (RPQ).Overall, these findings help elucidate the neural mechanisms of impaired cognition and behavior after mild TBI and also suggest resting state fMRI functional connectivity as a diagnostic and prognostic biomarker in these patients.

- Reliable outcome prediction in mild TBI is important for clinical practice. Identifying patients at increased risk of unfavorable outcome permits targeting closer observation and early intervention, which may reduce the psychological burden of injury on the patients, and the related economic burden on society. We have demonstrated that existing models for mild TBI perform unsatisfactorily. We tested 21 variables in ordinal analysis of 386 patients, which is 1 in 18 and thus reasonable from a statistical perspective. Although we have found some strong predictors of poor outcome, such as age and history of psychiatric condition, given the sample size, we consider the results of our prognostic analysis as hypothesis generating. These predictors will need further validation in ongoing prospective longitudinal studies, such as those that are part of the International TBI Research Initiative (Lingsma et al).
- In a broad range of patients with mild to severe TBI measurement of GFAP-BDP yielded a net benefit above clinical screening alone and a net reduction in unnecessary ED CT scans by 12 to 30%. Used in conjunction with other clinical information, rapid measurement of GFAP-BDP is useful in establishing or excluding the diagnosis of radiographically apparent intracranial injury throughout the spectrum of TBI. As an adjunct to current screening practices, GFAP-BDP may help avoid unnecessary CT scans without sacrificing sensitivity (McMahon).
- Together our computational models indicate that multidimensional outcomes are more sensitive to early predictors such as CT pathology than traditional GOS-E, suggesting that high-resolution combinatorial endpoint monitoring can contribute to improved prognostic and diagnostic outcome modeling.
- We have harnessed multidimensional analytics and ensemble machine learning approaches to multi-analyte assays of TRACK-TBI pilot samples. This contributed to the numerous publications listed below which have demonstrated significant associations between genetic phenotypes and outcomes when used in combination with ensemble analytic approaches.
- We undertook prognostic modeling with a novel approach based upon the multimodal Allen Human Brain Atlas which integrates anatomic, genomic and MRI data resulting in a full transcriptome associated with each MRI voxel. In addition we have annotations reflecting the anatomical region of each transcriptome and anatomical mapping to the Montreal Neurological Institute (MNI) brain atlas space. We projected the expression levels of ANKK1, COMT and APOE-E4 alleles into the MRI Topographical Data Analysis map to determine the anatomical locations of expression in the human brain. The 3 genes are expressed in different MRI coordinate locations suggesting that these distinct molecular pathways have distinct locations in the human brain. Combined with our observation that the ANKK1 T/T and COMT G/G show up in two different subpopulations of mild TBI, (both of which have higher rates of PTSD and lower GOSE) the results suggest that we would want to simultaneously look at both SNPs in a multiplexed biomarker array to have coverage of the TBI syndromic space to predict outcome.
- These studies demonstrate that TDA is a useful tool for identifying specific patient groups in a large, heterogeneous TBI clinical dataset such as the TRACK-TBI pilot, in order to cluster individuals based on multivariate phenotypes across the entire syndromic outcome space. In addition, the results demonstrate that TDA can be a powerful tool to identify robust predictors of different patient group recovery trajectories for future treatment planning and clinical decision making.
- In the context of the TDA project we created an open-access, de-identified and publically available version of the TRACK-TBI pilot dataset that can be accessed through supplemental data and metadata files from the recent publication reporting these results (Nielson et al., 2017).

- The TRACK-TBI pilot dataset and images are available in FITBIR and has resulted in several collaborations and additional publications outside of the scope of this project.
- Fourteen papers have been published for this project with the TRACK-TBI pilot dataset
- Most importantly the research combining data across clinical, biomarker, imaging and outcome domains provides valuable data for the design of future clinical trials and treatment for TBI.

5. CONCLUSIONS

We have demonstrated progress across all aims which are of significance to civilian and military TBI clinical care. We have successfully created an Information Commons for the TBI CDE domains across the injury spectrum from concussion to coma. We have developed more sensitive image analysis tools and imaging biomarkers to better diagnose and predict mTBI patients that will have an unfavorable outcome. We have also identified new genetic markers in the dopamine pathway that appear to contribute to unfavorable outcome after TBI. This opens the possibility of pre-deployment and pre-injury (sports and occupational) screening for individuals at risk for unfavorable outcome following TBI. We have also clearly established the multifactorial nature of TBI and the need for a combination of clinical, imaging and blood-based marker for the diagnosis and prediction of outcome.

The ability to integrate data across multiple domains of brain pathology, cognitive and psychological outcomes, clinical measures and blood-based biomarkers, combined with demographics and medical history is an important step towards unpacking the heterogeneity that exists in TBI patients, and potential therapeutic windows that may be explored further for more precise treatments that may improve outcome. Not only have we identified several genetic variants that are predictive of outcome following various severities of TBI that can be measured early after trauma in blood samples for precision therapy decision making in the future, but we have also refined a machine learning tool that enables precision phenotype patient maps to be created to better understand the full complexity of the multi-system complications that arise following TBI. The impact this may have includes the development of new therapies that target the downstream effects of the signaling cascades these genetic variants are involved in (e.g. cellular responses to stress and DNA damage, dopamine processing, etc), and the ability to rapidly and accurately distill out robust findings from very large datasets with these new machine learning tools.

6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Published Manuscripts

1. Cnossen MC, Winkler EA, Yue JK, Okonkwo DO, Valadka AB, Steyerberg EW, Lingsma HF, Manley GT; TRACK-TBI Investigators. Development of a prediction model for post-concussive symptoms following mild traumatic brain injury: A TRACK-TBI Pilot study. *J Neurotrauma*. 2017 Mar 27. Epub ahead of print. PMID 28343409.
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7. INVENTIONS PATENTS AND LICENSES

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8. REPORTABLE OUTCOMES

None.

9. OTHER ACHIEVEMENTS

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10. REFERENCES

None.

11. APPENDICES

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Post-concussive symptomatology following mild TBI

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ABSTRACT

Post-concussive symptoms occur frequently after mild traumatic brain injury (mTBI) and may be categorized as cognitive, somatic, or emotional. We aimed to: 1) assess whether patient demographics and clinical variables predict development of each of these three symptom categories, and 2) develop a prediction model for six-month post-concussive symptoms.

MTBI patients (Glasgow Coma Scale score 13-15) from the prospective multicenter Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Pilot study (2010-2012) who completed the Rivermead Post-Concussion Symptoms Questionnaire (RPQ) at six-months post-injury were included. Linear regression was utilized to determine the predictive value of candidate predictors for cognitive, somatic, and emotional subscales individually as well as the overall RPQ. The final prediction model was developed using Lasso shrinkage and bootstrap validation.

We included 277 mTBI patients (70% male, median age 42y). No major differences in the predictive value of our set of predictors existed for the cognitive, somatic, and emotional subscales, and therefore one prediction model for the RPQ total scale was developed. Years of education, pre-injury psychiatric disorders and prior TBI were the strongest predictors of six-month post-concussive symptoms. The total set of predictors explained 21% of the variance, which decreased to 14% after bootstrap validation.

Demographic and clinical variables at baseline are predictive of six-month post-concussive symptoms following mTBI, however these variables explain less than one-fifth of the total variance in outcome. Model refinement with larger datasets, more granular variables, and objective biomarkers are needed before implementation in clinical practice.

Key words: post-concussion symptoms; prediction model; traumatic brain injury

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INTRODUCTION

Traumatic brain injury (TBI) is a common and often debilitating injury. In the United States alone, at least 2.5 million people suffer TBIs annually, accounting for 52,000 deaths, 275,000 inpatient hospitalizations, and 1,365,000 emergency room visits.[1] Approximately 70-90% of all TBI is characterized as 'mild TBI' (mTBI) defined by a Glasgow Coma Scale (GCS) score of 13 to 15 upon admission to the emergency department (ED).[2] Many patients recover completely from mTBI in the ensuing weeks to months.[3, 4] However, in 5-30% of subjects with mTBI, neurologic, cognitive and/or neuropsychiatric symptoms persist up to one year post-injury, or longer.[5-8] Methodologies to predict those at greatest risk of incomplete recovery are limited, but are the subject of active research incorporating neuroimaging, patient demographics, and genetic polymorphisms. Data from any of these sources may portend poor recovery.[9-13]

Post-concussive syndrome (PCS) is a clinical term used to describe a constellation of post-traumatic symptoms which may be divided into the domains of cognitive (forgetfulness, poor concentration, or slowed processing speed), somatic (headaches, double or blurred vision, photo or phonophobia, dizziness, nausea, disrupted sleep habits, or fatigue) or emotional (irritability, depression, frustration or restlessness).[14-17] The International Classification of Diseases, tenth edition (ICD-10) states that a diagnosis of PCS should include a head injury "usually sufficiently severe to result in loss of consciousness (LOC)," as well as three or more subjective symptoms present for at least four weeks. Symptoms should cause significant clinical impairment.[18]

In civilian populations, estimates suggest that roughly 10-20% of patients experience PCS within six months following mTBI.[14] However, the complaints are non-specific and are also observed in patients with extra-cranial injuries; because systemic injuries often coexist with neurological injuries, accurate estimates of true prevalence of PCS are difficult to ascertain. The term is not without controversy - for instance, after being included in the DSM-IV as a research diagnosis, PCS has been removed as a standalone disorder from the DSM-5 in favor of "major or mild neurocognitive disorder due to TBI."[19] In addition, there is overlap between the diagnostic criteria for PCS and posttraumatic stress disorder (PTSD),[20] further complicating the diagnosis of PCS. Therefore, it has been suggested that mTBI sequelae are more accurately understood as "post-concussive symptoms" rather than PCS.[5, 21] Nevertheless, prior efforts to identify and create prediction models of post-concussive symptoms have relied on surveying the entire constellation of PCS rather than analyzing individual symptoms and/or domains.[22-24]

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The Rivermead Post-Concussion Symptoms Questionnaire (RPQ) is one validated metric to survey post-concussive symptoms, relying on self-report as to the presence and severity of 16 symptoms.[16, 17, 25] It has been widely utilized to characterize outcomes and formally endorse symptomatology across the acute and chronic phases following mTBI.[26-29] The RPQ is constructed of individual symptom domains: cognitive deficits, somatic complaints, and emotional complaints – as described above.[16] Thus, the RPQ permits separate analysis of potential predictors of post-concussive symptoms in each domain. As different domains likely reflect different etiological pathways, one hypothesis is that each domain may be differentially susceptible to patient-specific and clinical factors. Alternatively, these complaints may reflect more global processes and therefore may not demonstrate differential susceptibility. The predictors that overlap across domains (cognitive, somatic, and emotional), and the predictors specific to each domain, warrant further delineation. Utilizing the prospective multicenter Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) dataset,[30] we investigated whether cognitive, somatic, and emotional symptoms have different predictors, and whether multivariable prediction modeling using patient demographics and clinical variables can be successfully applied to identify those at greatest risk for suffering post-concussive symptoms following mTBI.

MATERIALS AND METHODS

This study was conducted and reported according to the criteria of the *Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis* (TRIPOD) statement.[31]

Study Design

The TRACK-TBI Pilot study was a multicenter, prospective observational study conducted at three Level I Trauma Centers in the United States: San Francisco General Hospital (SFGH), University of Pittsburgh Medical Center (UPMC), and University Medical Center Brackenridge (UMCB) in Austin, Texas using the National Institute of Neurological Disorders and Stroke (NINDS) TBI Common Data Elements (CDEs) version 1 (https://commondataelements.ninds.nih.gov/TBI.aspx). Eligible subjects were enrolled upon presentation to the ED through convenience sampling at all three sites between April 2010 and June 2012. Institutional Review Board approval was obtained at all sites. Informed consent was obtained for all participants prior to enrollment in the study. For participants unable to provide consent due to their injury, consent was obtained from their legally authorized representative. Participants were re-consented, if cognitively able, at later inpatient and/or outpatient study follow-up assessments. The current analysis focuses on post-concussive symptoms as measured by the RPQ; other outcome measures obtained at six months post-injury included the Glasgow Outcome Scale-Extended (GOS-E), Brief Symptom Inventory – 18 Item, Post-Traumatic Stress Disorder Checklist – Civilian Version, Trailmaking Test, and California Verbal Learning Test, Second Edition, as previously described.[30]

Patient Selection

Inclusion criteria for the TRACK-TBI Pilot study were adult patients (age ≥16 years) presenting to one of the participating Level I trauma centers suffering external force trauma to the head with sufficient indications to triage to clinically indicated head computed tomography (CT) scan within 24 hours of injury. There were no requirements for visible pathology on CT scan.[30] Exclusion criteria were pregnancy, comorbid life-threatening disease, incarceration, serious psychiatric and neurologic disorders that would interfere with outcome assessment, and non-English speakers due to limitations in participation with outcome assessments. For the present study, our analysis was restricted to the subset of patients with mTBI, defined by a GCS ≥13.

Measurements

To assess the presence/absence and severity of post-concussive symptoms, subjects completed the RPQ at six months following injury, in-person with trained study personnel, preceded by the Galveston Orientation and Amnesia Test to assess capacity. All study personnel were trained on outcome measure administration by a single neuropsychological outcomes coordinator from UPMC. As previously described, the RPQ is a sensitive and validated assessment tool for the presence of post-concussive symptoms[16, 17, 25-29] and is a "CORE" level NINDS TBI CDE.[32] It is comprised of questions directed toward the following 16 symptoms: headache, nausea or vomiting, dizziness, sensitivity to noise, disrupted sleep, irritability, frustration, fatigue, depression, impaired memory, poor concentration, slowed thinking, blurred or double vision, light sensitivity, and restlessness. Each symptom is rated on a 5-point scale to assess whether the symptom has been absent, no more of a problem, or a mild, moderate, or severe problem in the 24 hours prior to completing the questionnaire, compared to pre-injury. As recommended by previous research,[33] the scores 0 and 1 were collapsed into a single category, scored at 0 points. This resulted in a 4-point scale with the following categories: symptom is absent or no more of a problem (0), symptom is mild (1), moderate (2), or severe (3). The total score was determined by adding up all scores 0 to 3, which results in a minimum score of 0 and a maximum score of 48. Subject responses may then be clustered into distinct neuropsychiatric domains: (i) cognitive deficits (impaired memory, poor concentration, slowed thinking), (ii) somatic complaints (headaches, blurred or double vision, noise sensitivity, dizziness, nausea, sleep disturbances, fatigue) and (iii) psychological complaints (irritability, depression, frustration, restlessness).[16]

Selection of Candidate Predictors

A systematic literature search was performed using subheadings and text words in EMBASE and Google Scholar to identify systematic reviews and prior published prediction modeling developing studies that assessed predictors of post-concussive symptoms (or related outcomes) following mTBI (see Appendix A for the EMBASE search strategy). To maximize the potential application of a prediction model to clinical practice, candidate predictors not readily available in the ED or during initial clinical evaluation were excluded. The following were chosen as candidate predictors: age, gender, years of education, pre-injury seizures, pre-injury migraine or headache, pre-injury psychiatric disorders, blood alcohol level (BAL: > 80 mg/dl (U.S. legal limit); ≤ 80 mg/dl; not

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measured), GCS score, CT abnormalities (present; absent), posttraumatic amnesia (PTA; present; absent; not measured), LOC (present; absent; not measured), and extracranial injury. We further included whether subjects suffered a prior TBI per self-report as a potential candidate predictor. Prior TBI was assessed using the NINDS TBI CDEs version 1,[34] and classified as yes (with or without hospitalization) or no. Although not found in systematic reviews and previous prediction modeling studies, we hypothesized that deficits from repeated TBIs may be cumulative and thus may result in greater post-concussive symptoms burden. Information on candidate predictors was gathered through abstraction of medical records and from patient interviews during the index hospital visit.

Statistical Analyses

Baseline characteristics of the overall study population were reported as medians and interquartile ranges (IQR), and frequencies and percentages, for continuous and categorical variables respectively. To verify whether loss to follow-up resulted in possible bias, we compared baseline characteristics of included patients with those patients who had a missing six-month RPQ (n = 199), using the Pearson chi-square statistic for categorical variables and the Mann-Whitney *U* test for continuous variables. Missing data on candidate predictors were subsequently imputed with a single imputation technique, meaning that values for the missing data points were estimated in a regression model using all other predictor variables and outcomes as independent variables.

We described the RPQ total scale and subscales (mean, SD, range), and assessed the association between the RPQ total scale and subscales and functional outcome (as measured by the GOS-E) as well as intercorrelations between scales, using the non-parametric Spearman's rho correlation coefficient. We subsequently calculated Cronbach's alpha for the RPQ total scales and subscales as a measurement of internal consistency.

To calculate the effect of candidate predictors on the RPQ cognitive, somatic and emotional subscales, we used univariable linear regression models with the candidate predictor of interest as independent variable and the RPQ subscale as dependent variable. To assess the adjusted effect of candidate predictors, we used multivariable linear regression models with all candidate predictors as independent variables. Unstandardized ß's and p-values were reported. The ß coefficient indicates the change in outcome (points on the RPQ scale or subscale) for one unit change in the predictor variable. To enhance comparability of effect estimates for the different subscales, we

additionally calculated standardized ß coefficients. A standardized ß indicates the change in outcome in SDs, for one SD change in the predictor variable.

To assess whether the predictor effects differed across cognitive, somatic and emotional subscales, we tested for interaction between the predictors (summarized in the predicted values of the RPQ total scale) and the subscales. We created three rows per patient in the database: one with the cognitive outcome, one with the somatic outcome, and one with the emotional outcome. We subsequently fitted a random effects model with a random intercept for patient number, the predicted value of the total RPQ scale based on the full multivariable model, ”outcome type” and an interaction between ”outcome type” and predicted value.

We developed the final model by using the least absolute shrinkage and selection operator (Lasso) method. This method shrinks the ß-coefficients in order to obtain less extreme ßs to enhance the external validity of a prediction model.[35] Variables with ßs that are unstable are shrunk to zero and omitted from the model. It should be noted that Lasso shrinkage focuses on the overall fit rather than statistical significance of individual predictors. As a consequence, predictors with a p-value > 0.05 could still be included in the final model. External validity of the final model was further enhanced by performing bootstrap validation with 100 samples.

The interaction test, Lasso shrinkage, and bootstrap validation were analyzed with R (version 3.2.2.) using the *lme4,*[36] *penalized*[37] and *foreign*[38] packages. All other analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21. A *p*-value < 0.05 was considered statistically significant in all analyses.

Sensitivity Analyses

Although a prediction model with a linear outcome is statistically more appealing, models with a binary outcome variable are often preferred for clinical interpretation. We therefore performed multivariable logistic regression analysis with the variables obtained after Lasso shrinkage as independent variables and the dichotomized RPQ scale as dependent variable. For the dichotomization of the RPQ we utilized the eight symptoms mentioned in the ICD-10 criteria. Subjects were subsequently diagnosed with PCS if they meet three or more of the following symptoms: (1) headache, (2) dizziness, (3) fatigue, (4) irritability, (5) insomnia, (6) memory problems, (7) concentration issues, and (8) frustration or depression (in ICD-10 explained as reduced tolerance to stress, emotional excitement or alcohol). It should be recognized that the RPQ cannot be used to truly diagnose ICD-10

PCS since the RPQ is based on self-report rather than clinical examination and does not include information on symptom duration and clinical significant impairment. In addition, there is no consensus as to whether symptoms should be included if they are rated as "mild symptom or worse" or if they are rated as "moderate symptom or worse."[39] We therefore applied both classifications.

We further examined the influence of attrition on estimates of the predictors by simulating three scenarios:

1. The patients lost to follow-up have relatively favorable outcomes in comparison to those included in current study
2. The patients lost to follow-up have similar outcomes to those included in current study
3. The patients lost to follow-up have relatively unfavorable outcomes in comparison to those included in current study

For the first scenario, we simulated the outcome of those lost to follow-up by generating random numbers with the range 0-48 (possible scores on RPQ), a mean of 0.00 (25th percentile of those included), and a SD of 10.0 (actual SD of those included). For the second scenario, we simulated outcome of those lost to follow-up with the range 0-48, a mean of 5.0 (median of those included), and a SD of 10.0 (actual SD of those included). For the third scenario, we simulated outcome with the range 0-48, a mean of 15 (75th percentile of those included), and a SD of 10.0 (actual SD of those included). For simplicity, we did not predetermine the associations between predictors and attrition, while acknowledging that this may play a role in the influence of attrition on effect estimates.

RESULTS

Patient Characteristics

The TRACK-TBI Pilot study consisted of 580 TBI subjects, of whom 476 had mTBI (GCS 13-15); 277 subjects (58%) completed six-month RPQ assessment and were included in the current analysis (Figure 1). Included subjects had more years of education (median: 14) than those lost to follow-up (median: 13, $p < 0.01$). No other statistically significant differences existed between those included in the current analysis versus lost to follow-up (Table 1).

Median age for subjects in the current analysis was 42 years (Interquartile range 26-57y) and most (70%) were male. Half of the subjects (n = 141) sustained a traffic accident. Fifty-four percent (n = 147) reported a prior TBI, for which 88 were hospitalized. By ED triage, 38% were discharged home, 35% were admitted to the ICU or other monitored inpatient bed, 23% were admitted to the ward, and 4% went directly to the operating room.

At six-months post-injury, the mean RPQ score was 8.8 (SD = 10.0). Fifty-three percent (n = 147) reported at least three or more out of the eight symptoms defined for PCS by ICD-10 as 'mild or worse,' while 27% (n = 74) reported at least three of eight symptoms as 'moderate or worse.'

RPQ Scales

The RPQ cognitive, somatic, and emotional subscales, and the RPQ total scale all demonstrated a skewed distribution with the majority of patients having relatively lower scores (Table 2). Cronbach's alpha was > 0.80 for the subscales and the total scale, indicating adequate internal consistency.[40] The RPQ total scale and subscales demonstrated moderate correlation with the GOS-E at six-months post-injury (r -0.61 to -0.71; $p < 0.01$), indicating that higher RPQ scores were associated with worse functional outcome. Intercorrelations between subscales were moderate (r 0.63 to 0.76; $p < 0.01$).

Predictors of Cognitive, Somatic, and Emotional Post-Concussive Symptoms

The cognitive, somatic, and emotional subscales were significantly associated with years of education ($p < 0.01$), pre-injury psychiatric disorders ($p < 0.01$), and prior TBI ($p < 0.01$) in both univariable and multivariable linear regression analyses. Strengths of the effect sizes, illustrated with standardized ßs, were similar across the three different scales (Appendix B). In addition, age, pre-injury seizures, pre-injury migraine and headache, and CT

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abnormalities were significant predictors for one or more subscales (Table 3). The interaction test between the cognitive, somatic, and emotional outcome subscales and the predicted value of the RPQ total scale was not statistically significant (t = 0.54; SE = 0.02). This indicates that although some differences exist on an individual predictor level, overall predictor effects are similar for the three subscales. Hence, one prediction model using the RPQ total scale as the outcome measure of choice could be developed from the current dataset – which comprised the next phase of our analysis.

Prediction Model of Six-month Post-Concussive Symptoms

The RPQ total scale was significantly associated with years of education ($p < 0.01$), pre-injury seizures ($p = 0.03$), pre-injury migraine and headache ($p < 0.01$), pre-injury psychiatric disorders ($p < 0.01$) and prior TBI ($p < 0.01$) in univariable analyses. In a multivariable model, the variables years of education ($p < .01$), pre-injury psychiatric disorders ($p < 0.01$), and prior TBI ($p < 0.01$) were statistically significant. We applied Lasso shrinkage to obtain the final set of independent predictors and their shrunken ßs. After shrinkage, the occurrence and severity of persistent post-concussion symptoms (higher scores on the RPQ) were associated with older age, female gender, less years of education, a confirmed or unknown PTA, a confirmed or unknown LOC and the presence of pre-injury migraine and headache, pre-injury psychiatric disorders andprior TBI(Table 4). Comparison of the expected values of the scales with the actual scores resulted in an R^2 of 0.21, which decreased to 0.14 after bootstrap validation. The expected score on the subscales and total scale could be calculated for individual patients by using the regression formula (Table 4, footnote). An example of the calculation for two individual patients is displayed in Box 1.

Box 1. Two cases and their predicted score on the RPQ scale according to our prediction model

Case 1 : Male patient, 65 years, 23 years of education with pre-injury headache or migraine, a pre-injury psychiatric disorder, a prior TBI, LOC, and PTA.

Predicted value total RPQ scale after six months = 14.45 (intercept) + (0.74*0) + (0.05*65) + (-0.79*23) + (2.07*1) + (3.73*1) + (3.71*1) + (-0.47*0) + (-0.38*0) = 9.04 (95% CI: 4.57 – 13.50)

Case 2: Female patient, 30 years, 10 years of education with pre-injury headache or migraine, a pre-injury psychiatric disorder and no prior TBI, LOC and PTA

Predicted value total RPQ scale after six months = 14.45 (intercept) + (0.74*1) + (0.05*30) + (-0.79*10) + (2.07*1) + (3.73*1) + (3.71*0) + (-0.47*1) + (-0.38*1) = 17.45 (95% CI: 13.00 – 21.90)

Note. Expected scores can be calculated with the regression formula in the footnote of Table 4. The 95% Confidence interval can only be calculated with advanced statistical software.

Sensitivity Analyses

Multiple logistic regression analyses with the variables obtained after Lasso shrinkage resulted in the same set of predictors being statistically significant (PCS classified as ≥ 3 'mild or worse' symptoms: years of education OR = 0.84 (95% CI: 0.76 – 0.93), pre-injury psychiatric disorders OR = 2.05 (95% CI: 1.14 – 3.68), prior TBI OR = 2.94 (95% CI: 1.71 – 5.08); PCS classified as ≥ 3 'moderate or worse' symptoms: years of education OR = 0.87 (95% CI: 0.77 – 0.97), pre-injury psychiatric disorders OR = 3.24 (95% CI: 1.77 – 5.91), prior TBI OR = 2.08 (95% CI: 1.10 – 3.93)). Female gender was a statistically significant predictor of PCS classified as ≥ 3 'mild or worse' symptoms (OR 2.02; 95% CI: 1.11 – 3.68). The Areas under the Curve (AUCs) ranged from 0.74 to 0.76, indicating reasonable discriminative ability (Appendix C). We did not apply further model development (e.g., shrinkage, bootstrap validation), since our sample size was too small to develop a valid model with a binary outcome.

When analyzing different scenarios of attrition, the scenarios in which patients lost to follow-up had similar or more favorable outcomes did not result in major changes in effect estimates. However, in the scenario where patients lost to follow-up had relatively unfavorable outcomes, prior TBI was no longer a statistically significant predictor of six-month post-concussive symptoms, while age, GCS, and PTA became significant predictors.

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DISCUSSION

We developed a prediction model to predict six-month post-concussive symptoms following mTBI in a multicenter study with 277 subjects. Post-concussive symptoms were associated with older age, female gender, less education, pre-injury migraine or headache, pre-injury psychiatric problems, prior TBI, PTA, and LOC, of which years of education, presence of pre-injury psychiatric disorders and prior TBI were the most robust predictors. This set of predictors accounted for less than one-fifth of the variance in post-concussive symptoms.

Previous investigations have often reported that PCS is a multidimensional concept.[5, 16, 17, 33, 41, 42] Therefore, it has been hypothesized that the cognitive, somatic, and emotional RPQ subscales are differentially susceptible to predictor variables. We did not find a difference in the predicted probabilities of the total set of candidate predictors for the three subscales, and therefore, we developed one overall prediction model for six-month post-concussive symptoms using the RPQ total scale. This might indicate that post-concussive symptoms from different domains share etiological factors. However, we did find differences in the predictive ability for some predictors (age, pre-injury seizures, pre-injury migraine and headache, CT abnormalities) and the intercorrelations between the three subscales were modest. Therefore, confirmation of our findings in larger patient samples is necessary to confirm the adequacy of the total RPQ scale as an outcome variable in prognostic research. Our final prediction model has an R^2 of 0.21, which decreased to 0.14 after bootstrap validation. This indicates that less than one-fifth of the variation in post-concussive symptoms could be explained by the predictors in the model. Despite being low for a prediction model, this is consistent with previous studies examining predictors of post-concussive symptoms using the linear RPQ as an outcome measurement. For example, in a systematic review conducted by Silverberg et al.[43] R^2s ranged from 0.06 to 0.89 in six studies that used the RPQ as a continuous outcome measurement, and was only above 0.40 in two studies deemed at high risk of statistical overfitting.[43]

In prior systematic reviews, the most robust predictors of mTBI sequelae were gender, pre-injury mental health, early post-injury neurological functioning, and post-injury anxiety.[43, 44] Consistent with this, pre-injury mental health was also a significant predictor in our study. Patients with pre-injury psychiatric disorders are known to be vulnerable to recurrence of the psychiatric disorder[45] or the development of other psychopathology,[46] which might be triggered by a stressful or traumatic event such as mTBI. Other significant predictors in our study were years of education and prior TBI. Both of these were also candidate predictors in the prediction model developed

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by Stulemeijer et al.[24] but were not found to be statistically significant in their final model, which was confirmed by the systematic review of Silverberg et al.[43] Nevertheless, higher education is associated with return to work in several studies,[24, 47, 48] and highly educated people generally have improved coping skills, cognitive and financial reserves, and a wider social network to deal with possible consequences of mTBI. The influence of prior TBI on persistent post-concussion symptoms is less often studied. However, emerging basic science and clinical research on repetitive brain injury suggests that the deleterious effects of brain injury are cumulative.[49] Therefore, inclusion of a history of prior TBI is an important consideration for future work on post-concussive symptoms and other neuropsychiatric sequelae of TBI. The predictors age, gender, pre-injury migraine and headache, PTA and LOC also appeared in our final prediction model because they contributed to the overall model fit. It however should be noted that they were not statistically significantly associated with persistent post-concussion symptoms and their potential as predictors should therefore be examined in future studies.

In creating our prediction model, we attempted to methodologically overcome several of the shortcomings of prior work. Our set of candidate predictors was based on existing literature and was appropriately limited to not exceed the rule of thumb of a maximum of one candidate predictor for every ten cases,[50, 51] which limits the risk of statistical overfitting.[50, 52] Additionally, we used Lasso shrinkage and bootstrap validation to correct for model optimism, improving generalizability of the model.[50, 52] Third, we examined the influence of predictors on the three RPQ subscales and tested whether the total RPQ scale as an outcome variable was adequate. The use of the RPQ as a linear scale might also be regarded as a strength of our study. Since there is no clear cut-off point determining whether a patient should be diagnosed with PCS, dichotomization might result in an arbitrary difference between favorable and unfavorable outcome, limiting its potential for clinical practice. For example, in our study we found that two different classifications of PCS (i.e. PCS ≥ 3 'mild or worse' symptoms vs. PCS ≥ 'moderate or worse' symptoms) resulted in a prevalence difference of 26%. Further, dichotomization results in a loss of information and potentially overoptimistic results.[50] Large sample sizes are needed to prevent statistical overfitting in prognostic studies with a dichotomous outcome, especially when the prevalence of patients with the outcome of interest is relatively low. In our study, we would have needed a total of 599 patients to develop a prediction model with a binary outcome variable (PCS defined as ≥ 3 moderate symptoms or worse). On the other hand, models with a dichotomized outcome are clinically appealing since these models can directly estimate the

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risk of post-concussive symptoms. In addition, it might be more relevant for clinicians to predict a clinical significant problem (e.g., PCS) rather than predicting an increase on the RPQ scale. The latter may also necessitate clinically relevant cut-off points that are currently unavailable. To improve clinical interpretation, we created a model with a dichotomous outcome for clinical interpretation.

We note several limitations. First, there was a significant proportion of subjects lost to follow-up (42%). Although this percentage is similar to other prospective studies in mTBI research[24, 53, 54] and patients lost to follow-up did not differ from those who remained, we cannot exclude selection bias. Patients included in our sample may, for instance, differ from those not included on factors that were not measured, or on the severity of their post-concussive symptoms. To estimate the possible effect of attrition on our estimation of predictors, we performed sensitivity analyses in which we simulated scenarios where patients lost to follow-up had a more favorable, similar, or more unfavorable outcome compared with those included in our study. We did not find major differences in the predictive probability of our set of predictors in the scenarios where patients lost to follow-up had similar or more favorable outcomes than the included patients. This corroborated similar studies analyzing the influence of attrition on predictor estimates.[55, 56] However, in the scenario were patients lost to follow-up had less favorable outcomes, additional predictors were associated with post-concussive symptoms, while prior TBI, which is a strong predictor in this study, was no longer statistically significant. The effect of attrition on outcome should therefore be taken into account when interpreting the results of the current study. A second limitation is that our sample size is relatively small for the development of a prediction model.[57] Consequently, our study might not have been sufficiently powered to detect the significance of some of the candidate predictors and current regression coefficients might be relatively unstable.[52] Third, in the present study, the included mild TBI patients were relatively severely injured. For example, 34% of the patients had CT abnormalities, and the majority of patients had PTA and LOC. In addition, 35% of the patients were admitted to step-down beds or the ICU. The relative severity of our study population may have implications for the generalizability to other populations of mTBI patients. Given these limitations, the results of the current study should be considered preliminary; validation in an independent population is needed.

We chose to develop a model with baseline and clinical predictors that can be gathered during the ED visit to maximize the potential application of the model in clinical practice. The inclusion of post-injury characteristics

may be less useful as mTBI patients may not receive routine follow-up after leaving the ED.[58] However, since our model explained less than one-fifth of the variation in six-month post-concussive symptoms, additional variables are likely necessary to obtain more reliable predictions. Since early post-injury symptoms have been shown to associate highly with chronic symptoms,[43] the addition of these symptoms could substantially improve our prediction model. Ideally, two models could be developed, validated, and implemented in future ED practices. First, a model based on baseline and clinical characteristics collected at ED presentation with a high sensitivity should be developed. This model could select high-risk patients that should be seen at a follow-up appointment soon after their ED visit. Such a model could be based on current findings and could further be refined with larger datasets, more granular variables and objective biomarkers. At the follow-up appointment, early post-injury symptoms could be further investigated and added to the model. This second model could subsequently identify patients at risk for long-term sequelae, who should be prioritized for preventative or rehabilitative interventions.

CONCLUSION

Demographic and clinical variables at baseline predict post-concussive symptoms after mild traumatic brain injury, however these variables explain less than one-fifth of the total variance in outcome. Model refinement with larger datasets, more granular variables, and objective biomarkers are needed before implementation in clinical practice.

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FIGURE LEGEND

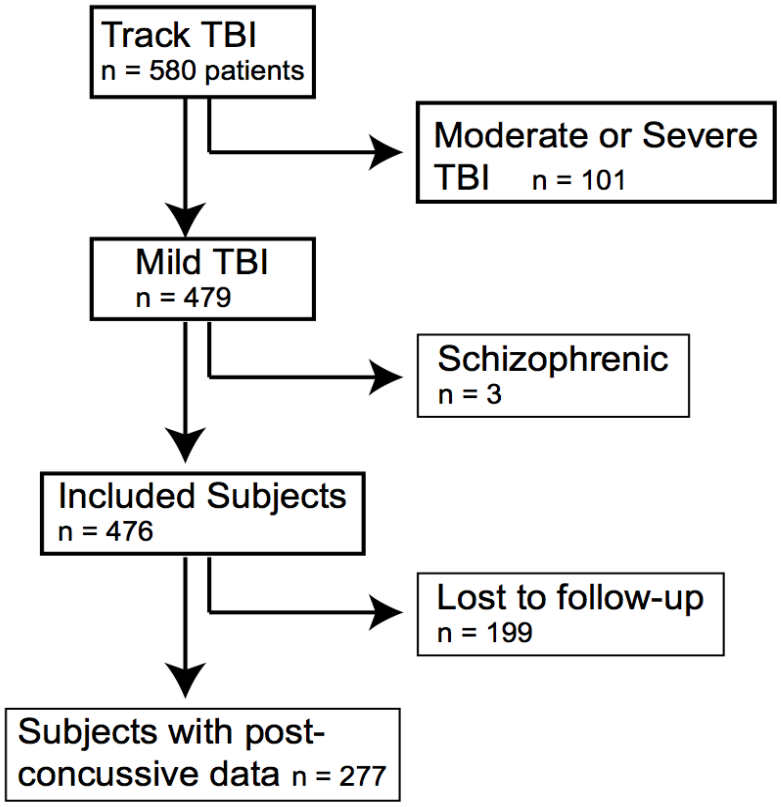

Figure 1. Flow-chart of included patients in current study

Development of a Prediction Model for Post-Concussive Symptoms following Mild Traumatic Brain Injury: A TRACK-TBI Pilot Study (doi: 10.1089/neu.2016.4819)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Note. Figure shows patients from the Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK TBI) pilot study that were included in current study

Table 1. Baseline characteristics of 277 subjects included in the study compared to 199 subjects lost to follow-up

	Included subjects (n = 277)		Subjects lost to follow-up (n = 199)		
Variable	**Missing**	**N (%)‡**	**Missing**	**N (%)‡**	***p*-value**
Age (median, IQR range)	-	42 (26-57)	-	43 (27-57)	.66
Gender (Female)	-	84 (30%)	-	51 (26%)	.26
Years of education (median, IQR range)	11	14 (12-16)	7	13 (12-15)	< .01
Pre-injury seizures*	-	30 (11%)	-	18 (9%)	.52
Pre-injury migraine & headache	-	36 (13%)	-	15 (8%)	.06
Pre-injury psychiatric disorders**	-	89 (32%)	-	49 (25%)	.08
Prior TBI	7	147 (54%)	14	84 (45%)	.06
Mechanism of injury	1		-		.11
- Traffic accident		141 (51%)		83 (42%)	
- Fall		84 (30%)		70 (35%)	
- Assault		39 (14%)		40 (21%)	
- Other		12 (5%)		6 (2%)	
BAL	-		-		.41
- ≤80 mg/dl (low BAL)		80 (29%)		53 (27%)	
- >80mg/dl (high BAL)		39 (14%)		37 (19%)	

- Not measured		158 (57%)		109 (54%)	
GCS < 15	-	63 (23%)	-	56 (28%)	.18
CT abnormalities***	-	95 (34%)	-	74 (37%)	.52
PTA	1		2		.38
- Yes or suspected		173 (63%)		112 (56%)	
- No		90 (32%)		72 (37%)	
- Unknown		13 (5%)		13 (7%)	
LOC	2		1		.58
- Yes		190 (69%)		132 (67%)	
- No		66 (24%)		55 (28%)	
- Unknown		19 (7%)		11 (5%)	
Extracranial AIS ≥ 3 in at least one body region	-	36 (13%)	-	32 (16%)	.34
ED disposition	-		-		.33
- Home		105 (38%)		62 (31%)	
- Hospital ward		63 (23%)		42 (21%)	
- Step-down bed or ICU		97 (35%)		88 (44%)	
- Operating room		12 (4%)		7 (4%)	

‡Values are presented as N (%) unless otherwise specified.

p-value presents results of Chi-Square test (categorical variables) or Mann-Whitney *U* test (continuous variables) for the differences between the included subjects and subjects that were lost to follow-up.

*Includes seizures and epilepsy

**Includes anxiety, depression, sleeping disorders and bipolar disorder

***Includes EDH, SDH, SAH, contusion, ICH, IVH, DAI, brain swelling, midline shift, cistern compression, fourth ventricle shift and third ventricle shift

Abbreviations. BAL = blood alcohol level; ED = emergency department; TBI = traumatic brain injury; GCS = Glasgow Coma Scale; CT = computed tomography; PTA = posttraumatic amnesia; LOC = loss of consciousness; AIS = abbreviated injury scale.

Table 2. RPQ outcome scales six months after mild traumatic brain injury

	Psychometric characteristics							Correlations		
	No. Items	**Mean**	**SD**	**Range**	**Possible range**	**Cronbach's alpha**	**GOSE**	**RPQ cognitive Scale**	**RPQ Somatic Scale**	**RPQ Emotional Scale**
RPQ Cognitive scale	3	2.25	2.74	0-9	0-9	.92	-.61*	-		
RPQ Somatic scale	9	4.32	5.34	0-27	0-27	.85	-.65*	.63*	-	
RPQ Emotional scale	4	2.19	3.07	0-12	0-12	.89	-.64*	.69*	.76*	-
RPQ Total scale	16	8.76	10.03	0-44	0-48	.93	-.71*	.82*	.94*	.90*

*p<.01

Results are presented after collapsing the RPQ scores 0 (no problem) and 1 (no more of a problem) together.

Correlation coefficients represent non-parametric Spearman's rho correlation coefficients.

Cognitive scale is based on the items forgetfulness, poor concentration and taking longer to think

Somatic scale is based on the items headache, dizziness, nausea, noise sensitivity, sleep disturbance, fatigue, blurred vision, light sensitivity and double vision

Emotional scale is based on the items irritability, depressed, frustrated and restlessness

Abbreviations. RPQ = Rivermead Post Concussion Questionnaire; SD = standard deviation; GOSE = Glasgow Outcome Scale Extended

Table 3. Univariable and multivariable predictors of cognitive, somatic and emotional post-concussive symptoms after six-months in 277 patients with mild traumatic brain injury

	RPQ – cognitive (3 items)		RPQ – somatic (9 items)		RPQ – emotional (4 items)	
Predictors	**Univariable (ß, p value)**	**Multivariable (ß, p value)**	**Univariable (ß, p value)**	**Multivariable (ß, p value)**	**Univariable (ß, p value)**	**Multivariable (ß, p value)**
Age (/10y)	0.15 (p = .09)	0.11 (p = .23)	0.30 (p = .10)	0.38 (p = .03)	0.02 (p = .83)	0.09 (p = .40)
Gender (Female vs Male)	0.39 (p = .27)	0.58 (p = .10)	0.74 (p = .29)	0.88 (p = .19)	0.05 (p = .90)	0.22 (p = .58)
Years of education (/y)	-0.24 (p < .01)	-0.23 (p < .01)	-0.46 (p < .01)	-0.39 (p < .01)	-0.24 (p < .01)	-0.21 (p < .01)
Pre-injury seizures* (yes vs. no)	1.14 (p = .03)	0.47 (p = .36)	1.85 (p = .07)	-0.001 (p = .99)	1.32 (p = .03)	0.44 (p = .46)
Pre-injury migraine & headache (yes vs. no)	0.86 (p = .08)	0.02 (p = .96)	4.58 (p < .01)	2.82 (p < .01)	1.50 (p = .01)	0.45 (p = .41)
Pre-injury psychiatric disorders** (yes vs. no)	1.57 (p < .01)	0.99 (p = .01)	3.14 (p <.01)	2.04 (p < .01)	1.67 (p <.01)	1.12 (p < .01)
Prior TBI (yes vs. no)	1.08 (p < .01)	1.19 (p < .01)	2.54 (p < .01)	2.04 (p < .01)	1.49 (p < .01)	1.10 (p < .01)
BAL						
-high BAL vs. low/unmeasured	-0.83 (p = .12)	-0.94 (p = .08)	0.50 (p = .64)	0.59 (p = .56)	0.15 (p = .81)	-0.14 (p = .81)
-unmeasured BAL vs. high/low	-0.67 (p = .07)	-0.59 (p = .11)	-0.09 (p = .91)	-0.17 (p = .81)	0.06 (p = .89)	-0.44 (p = .92)
GCS 13 or 14 vs GCS 15	0.17 (p = .67)	0.25 (p = .53)	0.24 (p = .75)	-0.14 (p = .85)	0.66 (p = .14)	0.52 (p = .25)
CT abnormalities*** (yes vs. no)	0.05 (p = .88)	0.46 (p = .22)	-1.32 (p = .05)	-0.35 (p = .62)	-0.90 (p = .02)	-0.42 (p = .33)

PTA						
- yes vs. no/unknown	-1.20 (p = .13)	-1.09 (p = .16)	0.74 (p = .63)	1.24 (p = .40)	-0.02 (p = .99)	-0.09 (p = .92)
- no vs. yes/unknown	-0.94 (p = .25)	-1.20 (p = .15)	0.51 (p = .75)	0.25 (p = .87)	- 0.10 (p = .92)	-0.42 (p = .65)
LOC						
- yes vs. no/unknown	0.36 (p = .59)	0.37 (p = .56)	-1.18 (p = .36)	-1.33 (p = .28)	-0.12 (p = .87)	-0.06 (p = .93)
- no vs. yes/unknown	0.02 (p = .98)	0.10 (p = .89)	-1.85 (p = .18)	-1.55 (p = .26)	-0.82 (p = .31)	-0.56 (p = .50)
Extracranial AIS ≥ 3 in at least one body region (yes vs. no)	-0.41 (p = .40)	-0.45 (p = .34)	-0.37 (p = .70)	-0.19 (p = .83)	-0.41 (p = .45)	-0.46 (p = .39)
R^2		0.20		0.23		0.17

Notes. Unstandardized ß's and p-values are shown for all analyses. The multivariable model is based on all candidate predictors in the table.

Cognitive scale is based on the items forgetfulness, poor concentration and taking longer to think

Somatic scale is based on the items headache, dizziness, nausea, noise sensitivity, sleep disturbance, fatigue, blurred vision, light sensitivity and double vision

Emotional scale is based on the items irritability, depressed, frustrated and restlessness

*Includes seizures and epilepsy

**Includes anxiety, depression, sleeping disorders and bipolar disorder

***Includes EDH, SDH, SAH, contusion, ICH, IVH, DAI, brain swelling, midline shift, cistern compression, fourth ventricle shift and third ventricle shift

Abbreviations. BAL = blood alcohol level; ED = emergency department; TBI = traumatic brain injury; GCS = Glasgow Coma Scale; CT = computed tomography; PTA = posttraumatic amnesia; LOC = loss of consciousness; AIS = abbreviated injury scale.

Table 4. Predictors of six month post-concussive syndrome in 277 patients with mild traumatic brain injury

Predictors	Univariable (ß, p value)	Multivariable (ß, p value)	LASSO shrinkage (ß)
Age (/10y)	0.50 (p = .16)	0.58 (p = .08)	0.53
Gender (Female vs Male)	1.18 (p = .37)	1.68 (p = .19)	0.74
Years of education (/y)	-0.94 (p < .01)	-0.84 (p< .01)	-0.79
Pre-injury seizures* (yes vs. no)	4.30 (p = .03)	0.91 (p = .63)	-
Pre-injury migraine & headache (yes vs. no)	6.95 (p < .01)	3.30 (p = .06)	2.07
Pre-injury psychiatric disorders** (yes vs. no)	6.28 (p < .01)	4.15 (p < .01)	3.73
Prior TBI (yes vs. no)	5.11 (p <.01)	4.34 (p < .01)	3.71
BAL			
-high BAL vs. low/unmeasured	-0.19 (p = .92)	-0.49 (p = .80)	-
-unmeasured BAL vs. high/low	-0.70 (p = .61)	-0.80 (p = .54)	-
GCS 13 or 14 vs GCS 15	1.07 (p = .46)	0.62 (p = .66)	-
CT abnormalities*** (yes vs. no)	-2.17 (p = .09)	-0.31 (p = .82)	-
PTA			
- yes vs. no/unknown	-0.47 (p = .87)	0.06 (p = .98)	-
- no vs. yes/unknown	-0.53 (p = .86)	-1.36 (p = .64)	-0.47
LOC			
- yes vs. no/unknown	-0.94 (p = .70)	-1.02 (p = .66)	-
- no vs. yes/unknown	-2.65 (p = .31)	-2.01 (p = .43)	-0.38
Extracranial AIS ≥ 3 in at least one body region (yes vs. no)	-1.20 (p = .51)	-1.09 (p = .52)	-

R^2		0.23	0.21ŧ

Notes. Unstandardized ß's and p-values are shown for all analyses. The multivariable model is based on all candidate predictors in the table.

The expected 6-months RPQ score can be estimated with the following formula:

6 month RPQ = 14.45 + (0.05*Age) + (-0.79*Years of education) + (0.74*female gender) + (2.07*pre-injury migraine or headache) + (3.73*pre-injury psychiatric disorder) + (3.71*prior TBI) + (-0.47* no PTA) + (-0.38*no LOC)

*Includes seizures and epilepsy

**Includes anxiety, depression, sleeping disorders and bipolar disorder

***Includes EDH, SDH, SAH, contusion, ICH, IVH, DAI, brain swelling, midline shift, cistern compression, fourth ventricle shift and third ventricle shift

Abbreviations. BAL = blood alcohol level; ED = emergency department; TBI = traumatic brain injury; GCS = Glasgow Coma Scale; CT = computed tomography; PTA = posttraumatic amnesia; LOC = loss of consciousness; AIS = abbreviated injury scale.

ŧR^2 decreased to 0.14 after bootstrap validation with 100 samples.

Appendix A. EMBASE search strategy

('brain injury'/exp OR 'brain injury assessment'/exp OR 'head injury'/exp OR concussion/exp OR (((brain OR head OR crani* OR intracrani* OR skull* OR cerebr* OR capitis OR hemisphere*) NEAR/3 (injur* OR trauma* OR posttrauma* OR damag* OR lesion* OR fracture*)) OR concus* OR contus* OR neurotraum* OR tbi OR mtbi):ab,ti) AND (injury/exp OR accident/exp OR emergency/exp OR 'emergency care'/exp OR 'emergency ward'/exp OR violence/exp OR (trauma* OR posttrauma* OR injur* OR tbi OR mtbi OR accident* OR emergen* OR violen*):ab,ti) AND ((mild* OR minor):ti,ab OR (mtbi OR mhi):ti,ab OR (concuss* NEAR/4 (symptoms OR syndrome*)):ti,ab OR (postconcuss* OR post-concuss*):ti,ab OR ((posttraum* OR post-traum*) NEAR/2 (symptom* OR complaint*)):ti,ab) AND ('prediction'/exp OR 'prognosis'/exp) OR ('prediction model' OR 'prognostic model' OR 'predictive model'):ti,ab NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Conference Paper]/lim OR [Editorial]/lim) AND [english]/lim NOT ([animals]/lim NOT [humans]/lim)

Appendix B. Standardized betas for univariable and multivariable regression analyses using the Rivermead Post concussion Questionnaire total scale and subscales as outcome measurement

	PCS – cognitive (3 items)		RPQ – somatic (9 items)		RPQ – emotional (4 items)		RPQ total scale (16 items)	
Predictors	**Univariable (standardized ß)**	**Multivariable (standardized ß)**	**Univariable (standardized ß)**	**Multivariable (standardized ß)**	**Univariable (standardized ß)**	**Multivariable (standardized ß)**	**Univariable (standardized ß)**	**Multivariable (standardized ß)**
Age (/y)	0.10	0.07	0.10	0.13	0.01	0.05	0.09	0.10
Gender (Female vs Male)	0.07	0.10	0.06	0.08	0.01	0.03	0.05	0.08
Years of education (/y)	-0.25	-0.24	-0.25	-0.21	-0.22	-0.20	-0.20	-0.24
Pre-injury seizures* (yes vs. no)	0.13	0.05	0.11	< 0.01	0.13	0.04	0.13	0.03
Pre-injury migraine & headache (yes vs. no)	0.11	< 0.01	0.29	0.18	0.17	0.05	0.23	0.11
Pre-injury psychiatric disorders** (yes vs. no)	0.27	0.17	0.28	0.18	0.26	0.17	0.30	0.19
Prior TBI (yes vs. no)	0.20	0.22	0.24	0.19	0.24	0.18	0.25	0.22
BAL								
-High BAL vs. low/unmeasured	-0.11	-0.12	0.03	0.04	0.02	-0.16	-0.01	-0.02
-Unmeasured BAL vs. high/low	-0.12	-0.11	-0.01	-0.02	0.01	-0.08	-0.04	-0.04
GCS 13 or 14 vs GCS 15	0.03	0.04	0.02	-0.01	0.09	0.07	0.05	0.03
CT abnormalities*** (yes vs. no)	0.01	0.08	-0.12	-0.03	-0.14	-0.07	-0.10	-0.02
PTA								
- yes vs. no/unknown	-0.21	-0.19	0.07	0.11	< 0.01	-0.01	-0.02	< 0.01
- no vs. yes/unknown	-0.16	-0.21	0.05	0.02	-0.02	-0.06	-0.03	-0.04
LOC								
- yes vs. no/unknown	0.06	0.06	-0.10	-0.12	-0.02	-0.01	-0.04	-0.05
- no vs. yes/unknown	< 0.01	0.02	-0.15	- 0.13	-0.11	-0.08	-0.11	-0.09
Extracranial AIS ≥ 3 in at least one body region (yes vs. no)	-0.05	-0.06	-0.02	-0.01	-0.05	-0.05	-0.04	-0.04

Notes. Standardized betas are shown for all variables. The multivariable model is based on all candidate predictors in the table.

*Includes seizures and epilepsy

**Includes anxiety, depression, sleeping disorders and bipolar disorder

***Includes EDH, SDH, SAH, contusion, ICH, IVH, DAI, brain swelling, midline shift, cistern compression, fourth ventricle shift and third ventricle shift

Abbreviations: PCS = post-concussive syndrome; TBI = traumatic brain injury; BAL = blood alcohol level; GCS = Glasgow coma scale; CT = computed tomography; PTA = posttraumatic amnesia; LOC = loss of consciousness; AIS = abbreviated injury score

Appendix C. Logistic regression analyses with predictors selected in Lasso shrinkage as independent variables and the Rivermead Post Concussion Questionnaire dichotomized using two different definitions as dependent variable

Variable	PCS defined as ≥ 3 out of 8 symptoms with score ≥ 2 (mild problem and worse)‡	PCS defined as ≥ 3 out of 8 symptoms with score ≥ 3 (moderate problem and worse)ǂ
	OR (95% CI)	OR (95% CI)
Age (/10y)	1.04 (0.89 – 1.22)	1.16 (0.97 – 1.39)
Gender (Female vs Male)	2.02 (1.11 – 3.68)	1.79 (0.93 – 3.43)
Years of education (/y)	0.84 (0.76 – 0.93)	0.87 (0.77 – 0.97)
Pre-injury migraine & headache (yes vs. no)	1.68 (0.70 – 4.07)	1.32 (0.57 – 3.02)
Pre-injury psychiatric disorders* (yes vs. no)	2.05 (1.14 – 3.68)	3.24 (1.77 – 5.91)
Prior TBI (yes vs. no)	2.94 (1.71 – 5.08)	2.08 (1.10 – 3.93)
PTA		
- yes vs. no/unknown	0.60 (0.16 – 2.23)	1.15 (0.28 – 4.69)
- no vs. yes/unknown	0.49 (0.12 – 1.98)	0.77 (0.17 – 3.21)
LOC		
- yes vs. no/unknown	1.28 (0.46 – 3. 59)	0.75 (0.25 – 2.25)
- no vs. yes/unknown	0.92 (0.29 – 2.92)	0.48 (0.13 – 1.78)
AUC	**0.74**	**0.76**

‡ 147 (53%) patients are diagnosed with PCS according to this definition
ǂ 74 (27%) patients are diagnosed with PCS according to this definition
* Includes anxiety, depression, sleeping disorders and bipolar disorder
Abbreviations: PCS = post-concussive syndrome; TBI = traumatic brain injury; PTA = posttraumatic amnesia; LOC = loss of consciousness; AUC = Area Under the Curve

Appendix D. The influence of attrition on the estimation of predictors

D1. Descriptions of three scenarios of attrition

Scenario	N	Mean score on Rivermead Post Concussion Questionnaire (SD)
Patients lost to follow-up not included in the analyses (analyses in this paper)	277	8.8 (10.0)
Scenario 1: Patients lost to follow-up have a relatively favorable outcome‡	476	6.6 (8.8)
Scenario 2: Patients lost to follow-up have an average outcome~~ł~~	476	7.7 (9.0)
Scenario 3: Patients lost to follow-up have a relatively unfavorable outcome~~ł~~	476	11.8 (10.4)

‡199 patients lost to follow-up received a random score on the Rivermead Post-Concussion Symptoms Questionnaire with a mean of 0.0 (25th percentile patients included at 6m follow-up) and a standard deviation of 10.0 (standard deviation patients included at 6-m follow-up)

~~ł~~ 199 patients lost to follow-up received a random score on the Rivermead Post-Concussion Symptoms Questionnaire with a mean of 5.0 (Median (50th percentile) at 6-m follow-up) and a standard deviation of 10.0 (standard deviation patients included at 6-m follow-up)

~~ł~~ 199 patients lost to follow-up received a random score on the Rivermead Post-Concussion Symptoms Questionnaire with a mean of 15 (75th percentile patients included at 6-m follow-up) and a standard deviation of 10.0 (standard deviation patients included at 6-m follow-up).

D2. Multiple regression analyses

Predictors	Scenario 1: Favorable outcome	Scenario 2: Average outcome	Scenario 3: Unfavorable outcome
Age (/y)	0.36 (p = .11)	0.43 (p = .06)	0.63 (p = .02)
Gender (Female vs Male)	1.10 (p = .22)	1.24 (p = .18)	1.21 (p = .27)
Years of education (/y)	-0.27 (p = .05)	-0.36 (p = .01)	-0.76 (p < .01)
Pre-injury Seizures* (yes vs. no)	1.36 (p = .31)	1.47 (p = .29)	-0.18 (p = .91)
Pre-injury Migraine & Headache (yes vs. no)	3.40 (p = .01)	3.28 (p = .02)	1.11 (p = .48)
Pre-injury psychiatric disorders** (yes vs. no)	2.99 (p < .01)	2.65 (p <.01)	2.56 (p = .02)
Prior TBI (yes vs. no)	2.73 (p < .01)	2.77 (p < .01)	1.04 (p = .30)
BAL			
-High BAL vs. low/unmeasured	-1.26 (p = .32)	-1.31 (p = .31)	-1.00 (p = .51)
-Unmeasured BAL vs. high/low	-1.37 (p = .14)	-1.51 (p = .11)	-1.14 (p = .30)
GCS 13 or 14 vs GCS 15	-0.33 (p = .72)	-0.25 (p = .79)	2.45 (p = .03)
CT abnormalities*** (yes vs. no)	-0.85 (p = .35)	-1.02 (p = .27)	-0.75 (p = .49)
PTA			
- yes vs. no/unknown	1.90 (p = .27)	2.16 (p = .22)	4.66 (p = .03)
- no vs. yes/unknown	1.07 (p = .56)	1.35 (p = .48)	3.18 (p = .15)
LOC			
- yes vs. no/unknown	-1.59 (p = .33)	-1.85 (p = .27)	-1.43 (p = .47)
- no vs. yes/unknown	-2.28 (p = .21)	-2.44 (p = .19)	-1.95 (p = .37)
Extracranial AIS ≥ 3 in at least one body region (yes vs. no)	-0.18 (p = .87)	0.14 (p = .91)	2.12 (p = .12)

*Includes seizures and epilepsy

**Includes anxiety, depression, sleeping disorders and bipolar disorder

***Includes EDH, SDH, SAH, contusion, ICH, IVH, DAI, brain swelling, midline shift, cistern compression, fourth ventricle shift and third ventricle shift

Abbreviations: PCS = post-concussive syndrome; TBI = traumatic brain injury; BAL = blood alcohol level; GCS = Glasgow coma scale; CT = computed tomography; PTA = posttraumatic amnesia; LOC = loss of consciousness; AIS = abbreviated injury score

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Screening for Post-Traumatic Stress Disorder in a Civilian Emergency Department Population with Traumatic Brain Injury

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Abstract

Post-traumatic stress disorder (PTSD) is a condition associated with traumatic brain injury (TBI). While the importance of PTSD and TBI among military personnel is widely recognized, there is less awareness of PTSD associated with civilian TBI. We examined the incidence and factors associated with PTSD 6 months post-injury in a civilian emergency department population using measures from the National Institute of Neurological Disorders and Stroke TBI Common Data Elements Outcome Battery. Participants with mild TBI (mTBI) from the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot study with complete 6-month outcome batteries ($n = 280$) were analyzed. Screening for PTSD symptoms was conducted using the PTSD Checklist-Civilian Version. Descriptive measures are summarized and predictors for PTSD were examined using logistic regression. Incidence of screening positive for PTSD was 26.8% at 6 months following mTBI. Screening positive for PTSD was significantly associated with concurrent functional disability, post-concussive and psychiatric symptomatology, decreased satisfaction with life, and decreased performance in visual processing and mental flexibility. Multi-variable regression showed injury mechanism of assault (odds ratio [OR] 3.59; 95% confidence interval [CI] 1.69–7.63; $p = 0.001$) and prior psychiatric history (OR 2.56; 95% CI 1.42–4.61; $p = 0.002$) remained significant predictors of screening positive for PTSD, while education (per year OR 0.88; 95% CI 0.79–0.98; $p = 0.021$) was associated with decreased odds of PTSD. Standardized data collection and review of pre-injury education, psychiatric history, and injury mechanism during initial hospital presentation can aid in identifying patients with mTBI at risk for developing PTSD symptoms who may benefit from closer follow-up after initial injury care.

Keywords: emergency department screening; post-traumatic stress disorder; traumatic brain injury

Introduction

Post-traumatic stress disorder (PTSD) is a condition associated with traumatic brain injury (TBI).[1–4] PTSD is considered a stressor-related disorder, characterized by intrusion, avoidance, negative alterations in cognition and mood, and alterations in arousal and reactivity following exposure to a traumatic event.[5] PTSD is found among those who have experienced all levels of TBI severity.[2] However, higher rates occur in individuals with mild TBI (mTBI), compared with those in a general trauma population[3,6–9] or among those with severe head injury.[2] It has been hypothesized that greater cognitive deficits associated with severe TBI protect against development of subsequent PTSD symptoms.[2] Among those with mTBI, younger individuals report more severe PTSD symptoms, compared with older subjects.[10]

PTSD is well-characterized among military personnel with a history of TBI and has been estimated to affect 32–66% of subjects with military-related TBI.[3] However, PTSD is not limited to military populations. Independent reports have estimated that PTSD occurs in 11–40% of civilians following TBI.[8,10,11] Prior work has identified that PTSD symptoms tend to emerge between 1–3 months following injury and peak around 6 months post-TBI.[2,6,8,12] Independent reports have begun to identify predictors of PTSD following TBI. These include a spectrum of risk factors present

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prior to TBI (pre-TBI), at the time of TBI, or following TBI (post-TBI). For example, a history of pre-existing psychiatric disease, (e.g., anxiety or depression), lower socioeconomic status, lower levels of education, prior trauma, and single marital status have been shown to confer risk for PTSD.[2,8,11,14] The incidence of PTSD varies with injury severity and mechanism of injury. For example, patients with mTBI and those who are assaulted have a greater risk of developing subsequent PTSD symptoms, compared with individuals with more severe TBI or those whose TBI results from motor vehicle accidents or falls. Duration of post-traumatic amnesia (PTA) and a positive toxicology study also appear to confer added risk.[2,3,8] Following brain injury, a lack of social support, increased life stress, poor health satisfaction ratings, and presence of disability are associated with risk of PTSD.[2,7,8,15–17] However, the relative contributions of each risk factor and consensus as to the most salient factors for the development of PTSD symptoms in a civilian population following TBI has yet to be established.

A relationship between development of PTSD and functional disability following TBI has been suggested. In military populations, self-reported concussive and PTSD symptoms after TBI was associated with disability at time of military discharge.[16] Similarly, soldiers evacuated following a blast injury resulting in TBI had greater disability, as measured by the Glasgow Outcome Scale-Extended (GOS-E), than those evacuated for other medical reasons.[17] There have been multiple reports of associations between lower GOS-E, depression, post-concussive symptoms, and PTSD in civilian[16] and military populations.[17,18] Specifically, up to 87% of service members with TBI meeting PTSD symptom screening criteria demonstrate concurrent moderate disability on the GOS-E (≤ 6).[17] While reports of moderate disability range from 13–23% after mTBI,[19,20] in civilian populations, the proportion of patients who develop PTSD symptoms and thus may benefit from symptom alleviation through PTSD-based therapy has yet to be characterized and/or validated.

Several limitations exist in current literature examining PTSD in the civilian population.[21,22] Studies frequently target the examination of the more accessible, more ''injured'' hospitalized patients, while excluding the evaluation of the concussed and ''walking wounded'' and their associated demographic and socioeconomic risk factors. Further, follow-up and comprehensive assessment of mTBI patients in the post-acute setting remains challenging and measures of PTSD symptomatology typically have not been included in standardized civilian outcomes batteries. In the current study, mTBI (Glasgow Coma Scale [GCS] score 13–15) patients were assessed with a broad range of outcome measures selected from the National Institute of Neurological Disorders and Stroke (NINDS) TBI Common Data Elements (TBI-CDE) Outcome Battery that included the PTSD Checklist-Civilian Version (PCL-C).[23–26] We report the incidence of PTSD symptoms at 6 months—a time when PTSD symptoms are reported to peak[3,27]—examine pre- and peri-injury risk factors, and describe associations with functional disability distinctive from other post-injury outcomes.

Methods

Participants

Participants were recruited from three Level I trauma centers as part of the multi-center, prospective Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Pilot study.[28] These trauma centers included San Francisco General Hospital (SFGH), San Francisco, California, University of Pittsburgh Medical Center (UPMC), Pittsburgh, Pennsylvania, and University Medical Center at Brackenridge (UMCB), Austin, Texas. Study protocols were approved by the institutional review boards at each participating center. Eligible patients for the TRACK-TBI Pilot study presented to the emergency department (ED) within 24 h of sustaining head trauma of sufficient severity to triage to a non-contrast head computed tomography (CT) scan using the American College of Emergency Physicians/Centers for Disease Control and Prevention evidence-based joint practice guidelines.[29] Informed consent was obtained from the patient or through proxy. Individuals who were non-English speakers, pregnant, in legal custody, or under a medically-evaluated psychiatric hold at the time of enrollment were excluded from the study.

Of 586 patients age ≥16 years enrolled in the TRACK-TBI Pilot study, a total of 338 completed the full 6-month TBI-CDE Outcome Battery, which included the PCL-C measure. Of these, 280 patients were classified as mTBI by ED admission GCS (13–15) and were included in the analysis. A higher number of study participants were enrolled at the SFGH site ($n = 196$), compared with at the UPMC ($n = 65$) and UMCB ($n = 19$) sites, and several differences in sample composition are noted. Specifically, SFGH had a higher proportion of participants with positive pre-injury psychiatric history ($p < 0.001$) and injury mechanism of assault ($p < 0.001$) and fewer Caucasian participants ($p < 0.001$; data not presented).

Measures

Demographic and injury characteristics were collected at the time of enrollment. The TRACK-TBI Pilot study outcome assessment battery listed below consisted of the core measures recommended by the NINDS consensus-based TBI-CDEs (Version 1).[23–26] Administered and self-reported neurocognitive and neuropsychological measures and global outcome ratings also were collected via in-person interview at 6 months post-injury.

Demographics. Data collected included age, race, gender, ethnicity, years of education, marital status, and employment status.

Baseline health status. Participants were queried according to TBI-CDE (Version 1) standard checklist of prior medical and psychiatric history.[23,24] This self-reported information was supplemented with data gathered through medical record abstraction.

Injury characterization. A variety of indices were collected to characterize TBI etiology and severity. These included GCS score[30] assessed by a neurosurgeon at hospital admission, duration of loss of consciousness (LOC), PTA, injury severity score (ISS),[31] hospital length of stay, discharge disposition, and location of discharge from the ED. Finally, CT scans were categorized as being positive or negative for acute intracranial lesions.

PCL-C. The PCL-C[25,32] is a standardized self-report rating scale of 17 PTSD symptoms that correspond to *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision* (DSM-IV-TR) criteria for PTSD across three component subcategories (''Re-experiencing,'' ''Avoidance,'' and ''Hypervigilance''), causing clinically significant distress or impairment for more than one month.[33] Respondents are asked to rate on a 5-point scale (1 = not at all to 5 = extremely) how much they have been bothered by each symptom in the past month. A higher score indicates more symptomatology of PTSD. Subjects simultaneously endorsing a score of ≥3 in one or more symptoms under ''Re-experiencing,'' three or more symptoms under ''Avoidance,'' and two or more symptoms under ''Hypervigilance'' subcategories on the PCL-C were coded as positive for PTSD.

GOS-E. The GOS-E[34] provides an overall measure of disability based on scales of cognition, independence, employability,

and social/community participation collected via structured interview. Individuals are described by one of the eight outcome categories: 1 = dead, 2 = vegetative state, 3 = lower severe disability, 4 = upper severe disability, 5 = lower moderate disability, 6 = upper moderate disability, 7 = lower good recovery, and 8 = upper good recovery. Good recovery is defined as a score of 7–8, moderate disability is defined by a score of 5–6, and severe disability is defined as a score of 3–4. A GOS-E score of 8 reflects full recovery to baseline status with no disability.

Brief Symptom Inventory 18. The Brief Symptom Inventory 18 (BSI18)[35] is used to assess psychological distress, with each item rated on a 5-point scale from 0 (not at all distressed) to 4 (extremely distressed). The Global Severity Index is represented by a T-score composed of the sum of three subscales—depression, somatization, and anxiety—containing six items each. Higher scores reflect greater psychological distress. An overall score of ≥63 meets the cutoff for clinical screening indicating a need for further assessment.

Rivermead Post-Concussion Symptom Questionnaire-13 Item. The Rivermead Post-Concussion Symptom Questionnaire-13 Item (RPQ-13)[36] queries the presence and severity of somatic, cognitive, and emotional symptoms that are commonly reported following TBI. Participants are asked to compare current (past 24 h) versus pre-injury symptom severity on a scale of 0 to 4 (0 = not experienced; 1 = no more of a problem; 2 = mild problem; 3 = moderate problem; 4 = severe problem). A score of ≥20 meets the cutoff for clinical screening for symptoms of post-concussion syndrome, a clinical state of persistent symptoms of a TBI.[37]

Satisfaction With Life Scale. The Satisfactions With Life Scale (SWLS)[38] is a global measure of life satisfaction consisting of five statements that the respondent is asked to endorse on a 7-point Likert scale (1 = strongly disagree to 7 = strongly agree). A higher score indicates greater life satisfaction. A score of >20 indicates some degree of satisfaction, and a score of <20 indicates some degree of dissatisfaction.

Trail Making Test. The Trail Making Test (TMT)[39] is a cognitive assessment consisting of two timed parts (TMT-A and TMT-B) that measure executive function and mental flexibility. Specifically, TMT-A assesses visual processing and TMT-B assesses mental flexibility and processing speed.

California Verbal Learning Test-Second Edition. The California Verbal Learning Test-Second Edition (CVLT-II)[40] is a verbal learning and memory task in which there are five learning trials, an interference trial, immediate (short-delay) recall trials, and post-20 min (long-delay) recall trials. The standard score (normalized for age, years of education, and handedness) for long-delay free recall was used in this analysis as a measure of encoded verbal memory.

Wechsler Adult Intelligence Scale-Fourth Edition, Processing Speed Index. The Wechsler Adult Intelligence Scale-Fourth Edition, Processing Speed Index (WAIS-PSI)[41] subscale is composed of the Symbol Search and Coding tasks, which require visual attention and motor speed. The scaled composite PSI score (normalized for age), which ranges from 50 to 150 to correspond to the 0.1 to 99.9 percentile of performance across age groups, was used in this analysis. Scores of ~90, 100, and ~110 correspond to the 25th, 50th, and 75th percentiles, respectively.

Statistical analysis

Patients who completed the 6-month PCL-C data were selected for this study ($n = 280$). PTSD status was determined by dichotomous classification on the PCL-C according to DSM-IV criteria based on the number and categories of symptoms reported.[33] Statistical analysis first examined differences in baseline variables, comparing participants who were positive for PTSD symptoms and those who were not. Differences in means and frequencies for continuous and categorical variables, respectively, were compared between those who screened positive for PTSD symptoms and those who did not screen positive at 6-month follow-up. Continuous variables identified as having a skewed distribution (Shapiro-Wilk W statistic <0.05) were compared using the Wilcoxon Mann-Whitney test. Categorical variables were compared using Pearson's chi-square test and Fisher's exact test for comparisons with group counts <5.

To further explore the association between potential relevant baseline predictors and positive screening for PTSD at 6 months, we selected possible predictors as identified from the literature[2,8,11,14] and from clinical knowledge, including demographics (age, gender, race, education, marital status), medical history, mechanism of injury, acute toxicology, head injury severity (CT, GCS), and overall injury severity (ISS). Baseline variables identified as having a significant association with PTSD in univariate analysis ($p < 0.05$) were selected to be included in a multi-variable logistic regression model predicting the probability of being diagnosed with PTSD based on the PCL-C scale. These variables included demographic, pre-injury, and injury-related variables, including race, years of education, marital status, prior psychiatric history, and injury mechanism (assault vs. all other causes). Caucasian race and married marital status were included as binary variables.

Other 6-month outcome measures were not included as independent variables in the model as the aim of the study was to examine baseline factors associated with PTSD. These measures were analyzed by comparing mean scores between those with and without a positive screen for PTSD to understand symptoms and conditions associated with PTSD at the time of follow-up.

A variable selection procedure was then applied to improve the performance of the initial non-parsimonious prediction model using a step-wise forward procedure (p-entry ≤0.25; p-remain ≤0.15) based on the Hosmer and Lemeshow goodness-of-fit statistic. The association between each potential predictor and the outcome is reported on the odds ratio scale, together with its 95% confidence intervals. The parsimonious model's goodness-of-fit is expressed using the c-statistic. All statistical analyses were run on SPSS v.21 (Chicago, IL).

Results

Of 280 patients included in the analysis, mean age was 42.9 years (standard deviation [SD] = 17.8) and 69.3% of patients were male. Mean years of education was 14.4 (SD = 2.9) and patients were predominantly Caucasian (81.8%).

Comparison to demographic variables at time of injury

Overall, 75 (26.8%) screened positive for PTSD symptom criteria at 6 months post-injury (PTSD-positive). The PTSD-positive group was less likely to be of Caucasian race (73.3% vs. 84.9%; $p = 0.027$), reported fewer years of education (13.5 vs. 14.7 years; $p = 0.002$), were less likely to be married (20.0% vs. 35.1%; $p = 0.015$), and had a higher incidence of self-reported pre-injury psychiatric disturbance (53.3% vs. 26.8%; $p < 0.001$) than the PTSD-negative group (Table 1). With regard to the index injury of enrollment, the PTSD-positive group included more victims of assault (33.3% vs. 8.8%; $p < 0.001$). A nonsignificant statistical trend of lower ISS was observed in the PTSD-positive group (7.3 ± 8.5 vs. 9.8 ± 10.4; $p = 0.062$). The PTSD-positive group

Table 1. Demographic and Injury Characteristics by 6-Month PTSD Status after Mild TBI

Characteristic	*Sample size (n = 280)*	*No PTSD (n = 205)*	*Yes PTSD (n = 75)*	*p*
Age, years (mean ± SD)	280	43.3 ± 18.8	42.0 ± 14.9	0.602
Gender *n* (%)	280			0.551
Male		140 (68.3)	54 (72.0)	
Female		65 (31.7)	21 (28.0)	
Race, Caucasian *n* (%)	280	174 (84.9)	55 (73.3)	0.027
Education, years (mean ± SD)	255	14.7 ± 2.8	13.5 ± 2.9	0.002
Marital status *n* (%)	280			0.017
Single		104 (50.7)	44 (58.7)	
Married		72 (35.1) [a]	15 (20.0) [b]	
Separated/divorced		13 (6.3) [a]	12 (16.0) [b]	
Widowed		7 (3.4)	3 (4.0)	
Other/unknown		9 (4.4)	1 (1.3)	
Married marital status	280			0.015
Married		72 (35.1)	15 (20.0)	
Not married		133 (64.9)	60 (80.0)	
Study *n* (%)	280			0.300
San Francisco General Hospital		138 (67.3)	58 (77.3)	
University of Pittsburgh Medical Center		52 (25.4)	13 (17.3)	
University Medical Center at Brackenridge		15 (7.3)	4 (5.3)	
Prior psychiatric history *n* (%)	280	55 (26.8)	40 (53.3)	<0.001
Military service history *n* (%)	280	31 (15.1)	4 (5.3)	0.039
Mechanism of injury *n* (%)	280			<0.001
MV (driver/passenger)		35 (17.1)	8 (10.7)	
MV (motorcyclist)		11 (5.4)	3 (4.0)	
MV (pedestrian/cyclist)		70 (34.1) [a]	14 (18.7) [b]	
Fall		60 (29.3)	25 (33.3)	
Assault		18 (8.8) [a]	23 (30.7) [b]	
Other		11 (5.4)	2 (2.7)	
Injury mechanism of assault	280			<0.001
Yes, mechanism of assault		18 (8.8)	25 (33.3)	
No, other mechanisms		187 (91.2)	50 (66.7)	
ED toxicology screen	280			0.044
Positive screen		7 (3.4)	7 (9.3)	
Negative screen		198 (96.6)	68 (90.7)	
Intracranial lesion on CT *n* (%)	280	95 (46.3)	30 (40.0)	0.345
ED admission Glasgow Coma Scale *n* (%)	280			0.254
13		6 (2.9)	2 (2.7)	
14		36 (17.6)	20 (26.7)	
15		163 (79.5)	53 (70.7)	
ED disposition *n* (%)	280			0.247
ED discharge		72 (35.1)	33 (44.0)	
Hospital admission		87 (42.4)	31 (41.3)	
Intensive care unit admission		46 (22.4)	11 (14.7)	
ISS (mean ± SD)	280	9.8 ± 10.4	7.3 ± 8.5	0.062
Injury severity *n* (%)	280			0.211
Minor/moderate injury (ISS <16)		140 (68.3)	57 (76.0)	
Moderate/severe/critical injury (ISS ≥16)		65 (31.7)	18 (24.0)	

[a] and [b] denote statistically significant subgroup differences. Number qualifying and proportions are shown for categorical variables. Means and standard deviations (SD) are shown for continuous variables.

PTSD, post-traumatic stress disorder; TBI, traumatic brain injury; SD, standard deviation; MV, motor vehicle; ED, emergency department; CT, computed tomography; ISS, injury severity score.

contained a lower proportion of persons reporting military service history for those with complete data (5.3% vs. 15.1%; $p = 0.039$).

Comparison to outcome measures at 6 months post-injury

At 6 months post-injury, the PTSD-positive group experienced higher levels of less than favorable outcome (GOS-E ≤ 6, 65.3% vs. 21.5%; $p < 0.001$), higher scores indicating psychological distress (BSI18, 66.8 ± 7.5 vs. 50.9 ± 9.7; $p < 0.001$), a higher rate of persistent post-concussive symptoms (RPQ-13, 26.8 ± 10.3 vs. 9.1 ± 9.3; $p < 0.001$), lower executive functioning and flexibility (TMT Part A time, 40.2 ± 21.3 vs. 33.2 ± 14.8 sec, $p = 0.004$; TMT Part B time, 104.4 ± 70.8 vs. 81.0 ± 50.7 sec, $p = 0.008$), lower verbal learning and memory (CVLT-II, –0.3 ± 1.3 vs. 0.1 ± 1.1; $p = 0.006$), lower nonverbal processing speed (WAIS-PSI, 96.5 ± 15.9 vs. 102.2 ± 14.9; $p = 0.009$) and lower satisfaction with life (SWLS, 15.2 ± 6.3 vs. 23.4 ± 7.4; $p < 0.001$; Table 2).

Table 2. Performance on Concurrent Outcome Measures by 6-Month PTSD Status after Mild TBI

Outcome measure	*Sample size (n = 280)*	*No PTSD (n = 205)*	*Yes PTSD (n = 75)*	*p*
Glasgow Outcome Scale-Extended (GOS-E): less than favorable outcome (GOS-E ≤ 6 vs. GOS-E ≥ 7) *n* (%)	280	34 (21.5)	49 (65.3)	<0.001
Brief Symptom Inventory 18 Global Severity Index T Score (mean ± SD)	278	50.9 ± 9.7	66.8 ± 7.5	<0.001
Rivermead Post-Concussion Questionnaire-13 (mean ± SD)	279	9.1 ± 9.3	26.8 ± 10.3	<0.001
Trail Making Test, Part A time, in sec (mean ± SD)	248	33.2 ± 14.8	40.2 ± 21.3	0.004
Trail Making Test, Part B time, in sec (mean ± SD)	247	81.0 ± 57.9	104.4 ± 70.8	0.008
California Verbal Learning Test-Second Edition, Long Delay Free Recall Standard Score (mean ± SD)	240	0.1 ± 1.1	−0.3 ± 1.3	0.006
Wechsler Adult Intelligence Scale-Fourth Edition Processing Speed Index, composite score (mean ± SD)	247	102.2 ± 14.9	96.5 ± 15.9	0.009
Satisfaction With Life Scale (mean ± SD)	276	23.4 ± 7.4	15.2 ± 6.3	<0.001

Number qualifying and proportions are shown for the GOS-E. Means and standard deviations (SDs) are shown for all other outcome measures.
PTSD, post-traumatic stress disorder; TBI, traumatic brain injury.

The frequency of PTSD symptoms reported at 6 months post-TBI was highest in patients with a GOS-E score of 5 (22 of 34; 64.7%), followed by GOS-E scores of 6 (25 of 52; 48.1%) and 7 (23 of 87; 26.4%). Patients identified as having a moderate disability (GOS-E scores of 5 and 6) on global outcome at 6 months accounted for 62.7% of all PTSD-positive patients in the study (Fig. 1).

To examine the co-occurrence of PTSD symptom reporting and other conditions at the time of follow-up, measures were categorized to a domain of mental health (BSI18), post-concussive symptoms (RPQ-13), and cognitive deficit (CVLT-II, WAIS-PSI). Clinical screening cutoffs were established by test administration guidelines for each measure: BSI18 ≥ 63, RPQ-13 ≥ 20, CVLT-II ≤ −2 SD, and WAIS-PSI ≤5th percentile. Patients meeting the clinical cutoff for each domain were classified as positive for that domain. In 62 PTSD-positive patients with a full outcome battery, only four (6.5%) had isolated PTSD. Participants were likely to have

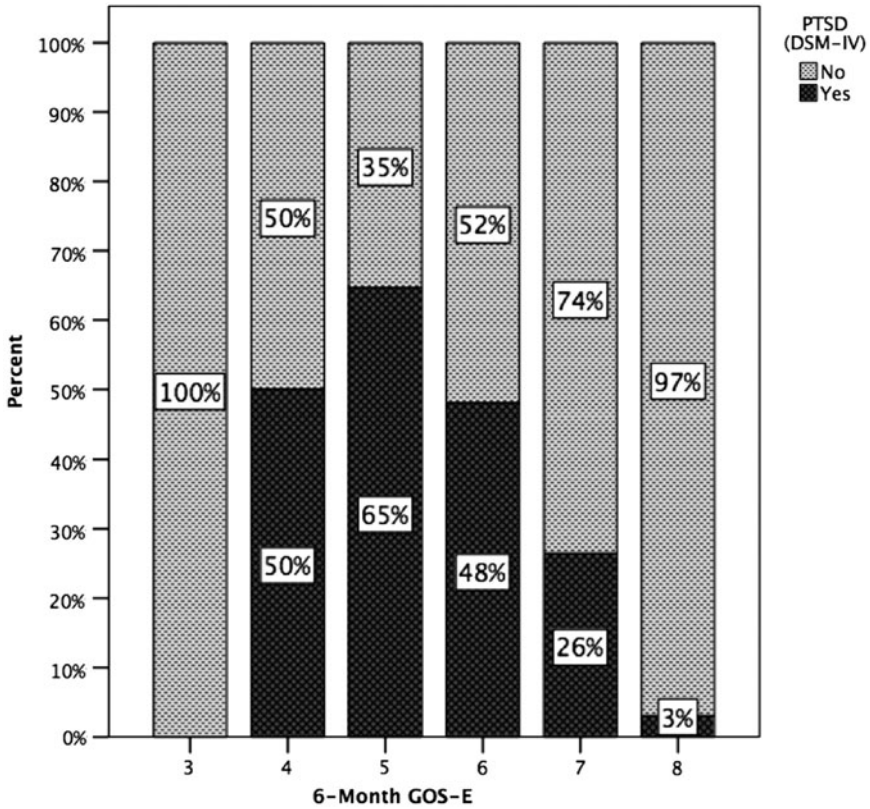

FIG. 1. Incidence of 6-month post-traumatic stress disorder (PTSD) after traumatic brain injury (TBI) within functional disability score categories. The proportion of subjects meeting screening criteria for PTSD by *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* (DSM-IV) criteria within each functional disability score category (Glasgow Outcome Scale-Extended [GOS-E]) is shown.

Table 3. Predictors of 6-Month PTSD after Mild TBI

Predictor	*B*	*OR*	*95% CI*	*p*
Caucasian race	–0.71	0.49	0.26–0.93	0.029
Education (per-year)	–0.16	0.85	0.77–0.94	0.002
Married marital status	–0.79	0.45	0.25–0.82	0.015
Prior psychiatric history	1.14	3.12	1.80–5.40	<.001
Mechanism of assault	1.53	4.60	2.31–9.15	<0.001

Univariate predictors with $p \leq 0.05$ for 6-month post-traumatic stress disorder by *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* criteria on binary logistic regression are shown.

PTSD, post-traumatic stress disorder; TBI, traumatic brain injury; OR, odds ratio; CI, confidence interval.

indicators of other conditions, including the presence of post-concussive and psychiatric disturbance, which accounted for the largest proportion (30 of 62; 48.4%), followed by psychiatric disturbance only (15 of 62; 24.2%), and then by all three coincident domains (8 of 62; 12.9%); three patients (4.8%) had cognitive impairment with PTSD, and two patients (3.2%) had only post-concussive symptoms with PTSD.

Logistic regression analysis confirmed the univariate predictive value of five baseline and clinical presentation variables: Caucasian race, years of education, marital status (married vs. all other), prior psychiatric history, and mechanism of assault. Reduced odds of screening positive for PTSD were associated with Caucasian race (OR, 0.49; 95% CI 0.26–0.93; $p = 0.029$), more years of education (per year OR, 0.85; 95% CI 0.77–0.94; $p = 0.002$), married marital status (OR, 0.45; 95% CI 0.25–0.82; $p = 0.015$). Increased odds of screening positive for PTSD were associated with prior psychiatric history (OR, 3.12; 95% CI 1.84–5.40; $p < 0.001$) and mechanism of assault (OR, 4.60; 95% CI 2.31–9.15; $p < 0.001$; Table 3) These five univariate predictors were selected for possible inclusion into the step-wise multi-variable logistic regression model. We did not assess the effect of CT pathology on PTSD as the focus of this analysis was the relationship between 6-month positive PTSD screen and baseline presentation.

Multi-variable analysis demonstrated that mechanism of assault (OR, 3.59; 95% CI 1.69–7.63; $p = 0.001$) and prior psychiatric history (OR, 2.56; 95% CI 1.42–4.61; $p = 0.002$) remained statistically significant predictors with increased odds of screening positive for PTSD at 6-months post-TBI. Education (per year OR, 0.88; 95% CI 0.79–0.98; $p = 0.021$) remained a statistically significant predictor, with decreased odds of screening positive for PTSD. The multi-variable model performed fairly (c-statistic, 0.713; 95% CI 0.642–0.785; $p < 0.001$) and conformed to goodness-of-fit (Hosmer and Lemeshow chi-square statistic 11.081; $p = 0.135$). Caucasian race and marital status did not persist as predictors after step-wise multi-variable analysis (Table 4).

Discussion

The frequency of participants screening positive for PTSD criteria among patients returning for follow-up in our study of mTBI was 26.8%, a prevalence that is consistent with prior reports of PTSD symptoms in civilian populations.[8,13] PTSD symptoms at 6 months post-injury rarely occurred in isolation. Rather, 94% of subjects with PTSD reported additional somatic, cognitive, and/or emotional symptoms. Analysis of the TRACK-TBI Pilot study data allowed the inclusion of patients traditionally excluded from previous hypothesis-driven research in the field, as pre-existing mental health conditions are common exclusion criteria. Incorporating educational history into the analysis led to the discovery that patients reporting PTSD symptoms at 6 months post-injury were more likely to have fewer years of education. Higher educational attainment previously has been shown to mitigate effects of moderate to severe TBI on cognitive status.[42] Although educational attainment was seen as a protective factor for reporting of PTSD symptoms, it is unclear if this finding was mediated by a relationship between educational status and cognitive outcomes.

This investigation revealed a high percent of individuals screening positive for PTSD (62.7%) in the moderate functional disability category by the GOS-E (score of 5 or 6). Studies assessing outcomes from individuals recruited in EDs typically do not use systematic approaches for ascertaining pre- and peri-injury mental health status.[43,44] Findings from Haagsma and colleagues identified an association with PTSD and functional disability measured by the GOS-E at 6 months follow-up,[18] consistent with the high proportions of patients screening positive for PTSD in the GOS-E 5 (64.7%) and GOSE 6 (48.1%) groups. Screening for PTSD, in conjunction with standardized examinations of pre-injury history at the time of initial medical care for TBI, could identify individuals who can benefit from more comprehensive follow-up.

Understanding the mechanism of injury is particularly important when considering the relationship between mTBI and PTSD. Previous studies suggest that individuals who sustain a TBI from intentional injuries are more likely to report PTSD symptoms and have poorer functional outcomes than other mechanisms of injury,[10,11,45] findings that are in agreement with the present study. However, the majority of our sample was enrolled from a single urban site which may not be representative of all patients with mTBI. Further examination of the relationship between pre-injury history, injury mechanism, and outcomes in individuals seeking care in urban emergency settings is warranted.

Estimating outcomes from TBI is complex. As recent reports indicate, behavioral variables may be more accurate in estimating functional outcomes of mTBI than injury severity ratings.[8,19,42] The relationship between PTSD symptom reporting and disability status following mTBI merits development of better PTSD clinical screening practices aimed at identifying patients and ameliorating

Table 4. Multivariable Predictors of 6-Month PTSD after Mild TBI

Predictor	*B*	*OR*	*95% CI*	*p*	*Model significance (p)*
Education (per year)	–0.13	0.88	0.79–0.98	0.021	<0.001
Prior psychiatric history	0.94	2.56	1.42–4.61	0.002	
Mechanism of assault	1.28	3.59	1.69–7.63	0.001	

For each iterative step, variables that did not achieve the pre-determined level of significance (p-entry ≤0.25) were not added to the model. Variables entered, but which did not remain significant within each iterative step (p-remain ≤0.15) were eliminated from the model (Caucasian race, married marital status). Three variables were ultimately included in the final model: education years, prior psychiatric history, and mechanism of assault.

PTSD, post-traumatic stress disorder; TBI, traumatic brain injury; OR, odds ratio; CI, confidence interval.

the impact of TBI and PTSD on long-term outcomes for individuals. Notably, in the current study, injury mechanism, psychiatric history, and education level persisted as independent risk factors after adjustment and thus underscores the importance of considering each demographic, socioeconomic, and event-of-injury characteristic during acute clinical evaluation of TBI. Conducting a more detailed patient history at the time of the initial injury and providing coordinated, multi-disciplinary care (e.g., social work, neuropsychology/psychiatry, rehabilitation) as recovery commences are practices reported to reduce PTSD symptoms and show promise for reducing long-term disability following TBI.[46]

Individuals who sustain a TBI and seek care in the ED are heterogeneous in clinical presentation, treatment, resources, and culture—all of which support the adoption of specific, relevant, and standardized data collection (TBI-CDEs) in order to: 1) accurately detect, characterize, and predict the incidence and/or development of PTSD after mTBI, and 2) converge data from multiple clinical sites with potentially distinct demographics and management practices for robust, reproducible, high-quality research to elucidate strategies to prevent or reduce PTSD symptoms after mTBI. In the TRACK-TBI Pilot study, education level, and incidence of baseline psychiatric history emerged as specific differences between those who reported PTSD symptoms throughout the 6-month recovery period among well-established urban Level I trauma centers. Hence, by implementation of the TBI-CDE Core Outcome Battery, we not only validate its utility, but also provide increased granularity of PTSD characterization and prediction, as well as provide support for previous findings that PTSD-like symptoms are indeed present following civilian mTBI. Given the approximately 27% incidence rate observed in this study, PTSD in civilian populations should be a topic of confirmatory and longitudinal analyses in the near future.

Study limitations

This study has several limitations. First, baseline medical history was collected primarily through self-report. Higher levels of granularity related to the patient's medical and psychiatric history at baseline, as well as the professional level of assessment (clinical cutoff points, self-report of symptoms) and the frequency of symptoms experienced, will yield more precision in identifying predictors for PTSD.

Second, a high frequency of participants who screened positive for PTSD were in a less than favorable outcome category even though they suffered mTBI based on GCS at admission. For the current study, we did not analyze comorbidities at the time of index TBI, such as polytrauma, which may contribute to or confound this finding. Future studies should explore the relationship of polytrauma and comorbidities at the time of injury to symptom reporting of post-injury outcomes.

Third, as this sample was not taken from a military population, military service history data were only applicable for a small proportion of the total sample (35 of 280), and the baseline assessment protocol did not include a detailed interview related to military service (e.g., number of years served, combat experience, and exposure to trauma during service). To better characterize the landscape of post-TBI PTSD in veterans within the civilian population, military service history data should be included in data standards for both TBI research and clinical care.

Fourth, injury mechanism of assault was a significant variable in the model. Although the subset of these individuals with intentional injury is small, TBI due to assault is associated with specific demographic factors. Individuals with intentional injuries are more likely to be male, non-Caucasian, single, and unemployed, and have lower levels of educational attainment, higher rates of intoxication, and a history of criminal behavior.[47–50] The connection between common factors of assault-related TBI and PTSD warrants further investigation, and in larger populations with more diverse demographic characteristics, location of medical care, and racial subgroups for validation.

Fifth, only 6.5% of patients screening positive for PTSD experienced it in isolation with respect to other psychiatric conditions. The high degree of coincidence of PTSD as defined by the stringent DSM-IV clinical criteria with multiple psychiatric, post-concussive symptom reporting, and neurocognitive outcome measures above their respective cutoffs for clinical screening suggests a multidimensional association of TBI and PTSD. Symptoms of PTSD and depression can overlap; indeed, in one study, subjects with major depressive disease reported comparable responses to as many classical PTSD items as patients who were diagnosed with PTSD.[51] In recent literature,[52] measures of cognitive effort were administered to validate cognitive and psychiatric symptoms. The current study utilizes measures arising from the TBI-CDEs, which currently do not include effort measures. As additive neurological dysfunction tends to overwhelm individual symptoms, current treatments for other domains of mental health may proportionally alleviate the behavioral burden of PTSD. As part of future research, collection and analysis of multi-dimensional psychiatric and cognitive measures, along with effort measures, may serve to alert the clinician to risks of developing PTSD during recovery and lead to earlier interventions during the subacute and chronic phases after TBI. Further study on larger populations will also likely reveal the contribution of pre-index injury factors, such as previous TBIs.

The sixth limitation is the use of DSM-IV[33] criteria in scoring the primary outcome measure of the study. The TRACK-TBI Pilot was completed prior to publication of the DSM-5, and the PCL-C was designed according to DSM-IV criteria for PTSD. Results from the current study await the augmentation and validation in future research using the PTSD Checklist for DSM-5 (PCL-5),[53] which corroborates the criteria of the DSM-5.

Conclusion

Expanding evidence supports the concept of TBI as a chronic disease characterized by delayed onset and possible progressive symptoms. In this study, positive screen for PTSD was identified in a large proportion of civilian patients 6 months following acute mTBI. Pre-injury demographic and socioeconomic status, prior psychiatric history, and assault mechanism emerged as risk factors for positive 6-month PTSD screen, and should be evaluated at time of injury to better identify those who may benefit from post-injury follow-up. In the civilian ED setting of predominantly mTBI, standardized data collection of these injury characteristics and pre-existing risk factors at the time of injury care may assist in identifying significant morbidity attributable to PTSD and development of therapeutic strategies that may reduce the psychiatric burden associated with TBI. Our findings support the necessity of increasing awareness of PTSD in the civilian TBI population and promoting more routine PTSD screening of mTBI patients who are still symptomatic 6 months after their injury.

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Author Disclosure Statement

No competing financial interests exist.

Appendix

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Circulating Brain-Derived Neurotrophic Factor Has Diagnostic and Prognostic Value in Traumatic Brain Injury

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Abstract

Brain-derived neurotrophic factor (BDNF) is important for neuronal survival and regeneration. We investigated the diagnostic and prognostic values of serum BDNF in traumatic brain injury (TBI). We examined serum BDNF in two independent cohorts of TBI cases presenting to the emergency departments (EDs) of the Johns Hopkins Hospital (JHH; $n = 76$) and San Francisco General Hospital (SFGH, $n = 80$), and a control group of JHH ED patients without TBI ($n = 150$). Findings were subsequently validated in the prospective, multi-center Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) Pilot study ($n = 159$). We investigated the association between BDNF, glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase-L1 (UCH-L1) and recovery from TBI at 6 months in the TRACK-TBI Pilot cohort. Incomplete recovery was defined as having either post-concussive syndrome or a Glasgow Outcome Scale Extended score <8 at 6 months. Median day-of-injury BDNF concentrations (ng/mL) were lower among TBI cases (JHH TBI, 17.5 and SFGH TBI, 13.8) than in JHH controls (60.3; $p = 0.0001$). Among TRACK-TBI Pilot subjects, median BDNF concentrations (ng/mL) were higher in mild (8.3) than in moderate (4.3) or severe TBI (4.0; $p = 0.004$. In the TRACK-TBI cohort, the 75 (71.4%) subjects with very low BDNF values (i.e., <the 1st percentile for non-TBI controls, <14.2 ng/mL) had higher odds of incomplete recovery than those who did not have very low values (odds ratio, 4.0; 95% confidence interval [CI]: 1.5-11.0). The area under the receiver operator curve for discriminating complete and incomplete recovery was 0.65 (95% CI: 0.52-0.78) for BDNF, 0.61 (95% CI: 0.49-0.73) for GFAP, and 0.55 (95% CI: 0.43-0.66) for UCH-L1. The addition of GFAP/UCH-L1 to BDNF did not improve outcome prediction significantly. Day-of-injury serum BDNF is associated with TBI diagnosis and also provides 6-month prognostic information regarding recovery from TBI. Thus, day-of-injury BDNF values may aid in TBI risk stratification.

Key words: biomarkers; brain-derived neurotrophic factor; glial fibrillary acidic protein; traumatic brain injury; ubiquitin C-terminal hydrolase-L1

Introduction

Diagnosis of traumatic brain injury (TBI) and early identification of patients at risk for long-term consequences of TBI represents a unique clinical challenge with major public health implications. A number of candidate circulating TBI biomarkers have shown promise for aiding in the diagnosis of TBI and in identifying patients with traumatic abnormalities on head computed tomography (CT) scan.[1–4] Importantly, their ability to predict adverse consequences of TBI has been limited. Objective diagnosis

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and prognosis of TBI will help improve triaging to appropriate medical care at time of injury, guide judicious use of neuroimaging, and inform the development of ''return to work or play'' guidelines. Additionally, while most patients with mild TBI (mTBI) recover to their pre-injury state within 3 months, a significant minority do not. Prognostic biomarkers that identify patients unlikely to make a full recovery are needed to identify appropriate candidates for clinical trials of novel TBI therapies.[5]

Brain-derived neurotrophic factor (BDNF), a member of the family of neurotrophic proteins, is a secreted autocrine factor that promotes the development, maintenance, survival, differentiation, and regeneration of neurons.[6,7] It is also important for synaptic plasticity and memory processing.[8,9] BDNF has been implicated in reducing secondary brain injury, with elevations providing neuroprotection and restoring connectivity after TBI.[10–12] However, the diagnostic and prognostic significance of day-of-injury circulating BDNF concentration are not well understood. We conducted a study to establish the association between BDNF and TBI and to determine whether day-of-injury BDNF values are associated with TBI severity and outcomes.

Glial fibrillary acidic protein (GFAP) is an astrocytic protein whose functions include cell communication, mitosis, and maintaining the integrity of the blood–brain barrier (BBB).[13] GFAP has excellent specificity for TBI-associated intracranial hemorrhage and focal mass lesions.[14,15] Elevated values are associated with increased mortality.[1,16]

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is a neuronal protein that is involved in the addition and removal of ubiquitin proteins flagged for metabolism. UCH-L1 is especially elevated in TBI and has been found to be associated with mortality.[17–19]

Methods

BDNF serum concentrations were determined in duplicate in two independent cohorts of TBI cases presenting to the Johns Hopkins Hospital (JHH) and the San Francisco General Hospital (SFGH) emergency departments (ED), and one control cohort of JHH ED patients presenting for non-TBI complaints. Findings were subsequently validated in the prospective, multi-center Transforming Research and Clinical Knowledge in TBI Pilot (TRACK-TBI) Pilot study.[20] We also compared the prognostic value of BDNF to that of two well-studied TBI biomarkers, GFAP and UCH-L1, since these biomarker values were available from a previous study.[18] Study protocols were approved by the institutional review boards at participating sites.

Study population

Case cohorts. JHH and SFGH are academic, tertiary care, Level 1 trauma institutions. Patients were eligible for inclusion as TBI cases if they presented to JHH and SFGH ED after experiencing acute blunt head trauma and met the following criteria: age 18 years or older; presented within 24 h of injury; met the American College of Emergency Physicians (ACEP) criteria for obtaining head CT scans in TBI[21]; received a non-contrast head CT scan as part of their clinical evaluation; and had excess serum sample available in the clinical chemistry lab. Cases met the definition of TBI proposed by the Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements (TBI CDE) for Research on Traumatic Brain Injury and Psychological Health.[22] Eligible cases were excluded if they had one of the following prior medical conditions: demyelinating disease, neurodegenerative disease, dementia, stroke, brain tumor, intracranial surgery, or active cancer. TBI cases were selected between November 2012 and September 2013. Since we utilized excess clinical blood samples, informed consent was waived.

Control cohort. Patients included as control subjects were JHH ED patients who were evaluated for suspected acute coronary syndrome,[23] had no blunt head trauma in the preceding 7 days, and were deemed to have a non-cardiac condition and discharged home from the ED. Eligible control subjects were excluded if they met any of the exclusion criteria for cases (see above). Control subjects did not receive head CT scans since there was no clinical indication for doing so. Clinical and demographic data were collected via structured patient interviews and a review of the electronic medical record. Subjects were enrolled between January 2012 and February 2013. Written informed consent was obtained from all subjects.

Validation cohort. The TRACK-TBI Pilot study enrolled subjects 16 years and older who presented to SFGH ED, the University of Pittsburgh Medical Center (UPMC) ED, and the University Medical Center Brackenridge (UMCB), Austin, TX, ED with TBI.[20] Patients were included in the study if they presented to the ED within 24 h of acute blunt force head trauma and met the ACEP criteria for obtaining a head CT in TBI, as previously described.[20] Only subjects from TRACK-TBI Pilot who had serum samples available for testing were included in the present study. Subjects in the validation cohort were enrolled from April 2010 to June 2011, and were distinct from those in the SFGH case cohort. Written informed consent was obtained from all subjects prior to enrollment in the study. Subjects unable to provide consent due to their injury were consented through their legally authorized representative at time of injury, and re-consented if cognitively able during their inpatient stay and/or their follow-up assessment time-point.

Serum sample collection and biomarker measurement

For the JHH and SFGH TBI case cohorts, excess serum samples stored in a 4°C refrigerator were retrieved from their respective clinical chemistry laboratory and stored in a − 80°C freezer. These samples were kept at 4°C for variable duration (median of 5 days). Serum samples for JHH control subjects and for TRACK-TBI Pilot subjects were collected, processed and stored in a − 80°C freezer within 2 h of collection, as previously described.[23,24] Samples for TRACK-TBI patients were collected within 24 h of injury.[13]

Samples were randomized and BDNF assayed in batches with an electrochemiluminescent sandwich immunoassay and read with a Sector Imager 2400 (Meso Scale Discovery, Rockville, MD). BDNF assay capture (MAB848) and detection antibodies (MAB648) and assay standard (248BD005) were obtained from R&D Systems (Duoset reagents, Cat. # DY248; Minneapolis, MN). Assays were performed within a single laboratory by staff blinded to clinical outcomes or study cohort. Samples from the different cohorts were shipped to this single academic laboratory. The assay lower limit of detection (LOD) was 0.0125 ng/mL and the lower limit of quantification was 0.5 ng/mL. As specified by the manufacturer, these assay reagents have no overlap with the TrK receptor proteins B-NGF, GDNF, NT-3, and NT-4. Assays were performed in duplicate. A previous study examining the stability of BDNF in blood samples stored at room temperature for 0-24 h, 24-48 h, 48-72 h, or > 72 h revealed an average increase of 1.67 (95% CI: 1.08-2.26) ng/mL per each 24-h period.[25] Since BDNF values are high in healthy subjects and low in diseased subjects, we defined low BDNF values as values that are lower than the 1st percentile in JHH non-TBI control subjects. This is analogous to the use of the 99th percentile as the recommended cut-off value in cardiac biomarkers[26,27] (values are high in diseased and low in healthy subjects).

GFAP and UCH-L1 were previously measured in TRACK-TBI Pilot in a single laboratory (Banyan Biomarkers, Alachua, FL).[15,18] The LOD of GFAP and UCH-L1 were 0.1 ng/mL and 0.03 ng/mL, respectively.

Table 1. The Department of Defense/Department of Veterans Affairs Classification of TBI Severity

Criteria	*Mild*	*Moderate*	*Severe*
Head CT/MRI	Normal	Normal/ abnormal	Normal/ abnormal
Loss of consciousness	0-30 min	> 30 min and < 24 h	> 24 h
Alteration of consciousness/mental state	< 24 h	> 24 h	> 24 h
Post-traumatic amnesia	< 1 day	> 1 and < 7 days	> 7 days
Best Glasgow Coma Scale score within first 24 h	13–15	9–12	<9

TBI, traumatic brain injury; CT, computed tomography; MRI, magnetic resonance imaging.

Outcomes

All patients enrolled in TRACK-TBI Pilot received head CT scans at the time of presentation to the ED. Each head CT was de-identified and read by a blinded board-certified neuroradiologist following the recommendations of the TBI-CDE Neuroimaging Working Group.[28] Our primary outcome, incomplete recovery at 6 months, was defined as a composite outcome of either post-concussive syndrome (PCS) or Glasgow Outcome Scale Extended (GOSE) score of <8 at 6 months, as these two measures together encompass a wider spectrum of the entire sphere of post-TBI outcomes. We defined PCS as having three or more symptoms on the 6-month Rivermead Post-Concussion Questionnaire[29] that were rated as worse than before the injury (score of 2).[30] The GOSE categorizes recovery after TBI on a scale of 1–8, where 1 = dead and 8 = upper good recovery. GOSE < 8 signifies incomplete recovery.[31] Additionally, head CT findings were classified as traumatic lesion present (this does not include isolated skull fractures) or no traumatic lesion present. TBI severity was classified as mild, moderate, or severe based on the Department of Defense/Department of Veterans Affairs definition (Table 1).[32]

Statistical analyses

Clinical and demographic data were summarized with descriptive statistics and differences were examined using the Mann-Whitney test (2-groups), the Kruskal-Wallis test (n-groups) and the χ^2 test (proportions). We quantified the discriminative ability of BDNF to distinguish between cases and controls, and to distinguish between TBI patients with relevant clinical outcomes and those without using area under the receiver operator curve (AUC). We also constructed logistic regression models to evaluate the association between BDNF values and clinical outcomes. We compared the AUCs of combinations of BDNF, GFAP, and UCH-L1 for discriminating between relevant clinical outcomes, using the method suggested by DeLong and colleagues.[33] This is a widely cited and generally accepted method that provides the confidence interval and standard error of the difference between two (or more) correlated AUCs.

To understand the determinants of BDNF in the control population, we constructed univariable and multi-variable linear regression models. Variables included in the models (age, gender, race, blood pressure, history of hypertension, history of depression or schizophrenia)[34–37] were selected based on an *a priori* literature

Table 2. Demographic and Clinical Characteristics of Study Population

	JHH non-TBI controls n = *150*	*JHH TBI cases* n = *76*	*SFGH TBI cases* n = *76*	*TRACK-TBI pilot cases* n = *159*	p *value*
Median age in years (IQR)	54 (47 – 62)	47 (30 – 56)	42 (26 – 56)	41 (25 – 56)	<0.001
Female (%)	79 (52.7)	29 (38.2)	22 (29.0)	45 (28.3)	<0.001
Race (%)					<0.001
• African-American	116 (77.3)	41 (54.0)	5 (6.6)	15 (9.5)	
• White	30 (20.0)	25 (32.9)	59 (77.6)	132 (83.5)	
• Other	4 (2.7)	10 (13.2)	12 (15.8)	11 (7.0)	
Mechanism of injury (%)					0.003
• Assault		19 (25.0)	13 (17.1)	23 (14.6)	
• Fall		26 (34.2)	23 (30.3)	50 (31.6)	
• MVC		21 (27.6)	11 (14.5)	51 (32.3)	
• Pedestrian struck		4 (5.3)	14 (18.4)	9 (5.7)	
• Struck by/against		3 (4.0)	2 (2.6)	5 (3.2)	
• Other trauma		3 (4.0)	13 (17.1)	20 (12.7)	
Glasgow Coma Scale (%)					0.09
• 3-8		5 (6.6)	4 (5.4)	19 (12.0)	
• 9-12		3 (4.0)	4 (5.4)	6 (3.8)	
• 13		2 (2.6)	3 (4.0)	1 (0.6)	
• 14		11 (14.5)	20 (27.0)	22 (13.8)	
• 15		55 (72.4)	43 (58.1)	111 (69.8)	
Traumatic intracranial abnormality on head CT (%)		21 (27.6)	24 (31.6)	75 (47.2)	0.006

JHH, Johns Hopkins Hospital; TBI, traumatic brain injury; SFGH, San Francisco General Hospital; TRACK-TBI, Transforming Research and Clinical Knowledge in TBI study; IQR, interquartile range; MVC, motor vehicle collision ; CT, computed tomography.

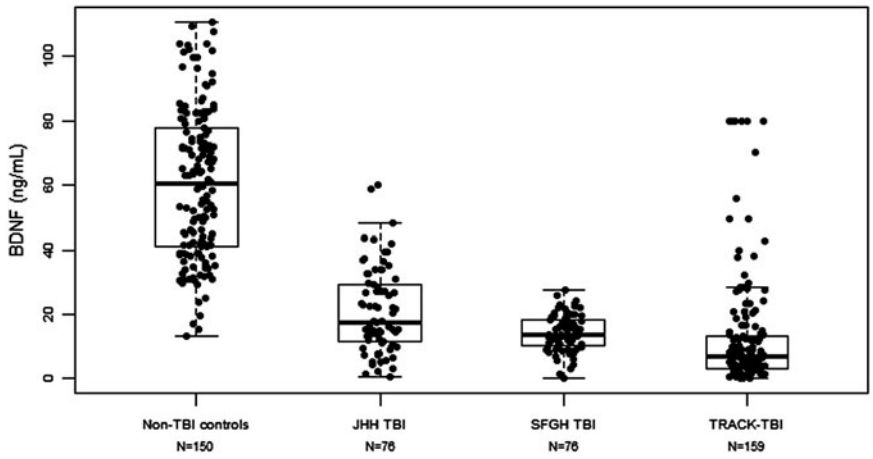

FIG. 1. Distribution of brain-derived neurotrophic factor (BDNF) in traumatic brain injury (TBI) and non-TBI cohorts. Graphical distribution of individual BDNF values and the corresponding box plots for Johns Hopkins Hospital (JHH) non-TBI control subjects, JHH TBI cases, San Francisco General Hospital (SFGH) TBI cases and Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) Pilot cases.

review. A two-tailed p value of < 0.05 was considered statistically significant. Statistical analyses were performed using STATA/MP statistical software version 11.2 (StataCorp, College Station, TX), and RStudio statistical software version 0.97.312 (Boston, MA).

Results

A total of 311 TBI cases were analyzed: 76 cases in the JHH TBI cohort, 76 cases in the SFGH TBI cohort, and 159 cases in TRACK-TBI Pilot, in addition to 150 JHH non-trauma control subjects. Non-trauma control subjects were older and more likely to be female or African-American, compared with TBI cases (Table 2).

Association between BDNF and TBI

In the initial case-control study, median day-of-injury BDNF values (ng/mL) were lower among TBI cases (17.5; interquartile range [IQR], 11.3-29.6) in JHH TBI group and 13.8 (IQR, 10.1-18.3) in the SFGH group) than in non-TBI controls (60.3; IQR, 41.1-78.2; $p = 0.0001$). The 1st percentile of BDNF values in JHH non-TBI controls was 14.2 ng/mL. BDNF discriminated between TBI cases (JHH and SFGH) and non-TBI controls with an AUC of 0.96 (95% CI: 0.94-0.98), which is considered excellent accuracy. There was no significant association between duration of storage of serum samples in 4°C and BDNF value among TBI cases (Supplementary Fig. 1; see online supplementary material at www.liebertpub.com). Similarly, in a validation study, median day-of-injury BDNF values (ng/mL) were found to be low among TRACK-TBI Pilot subjects (6.8; IQR, 3.0-13.5). The distribution of BDNF values among the TBI cases and non-TBI control subjects studied is presented in Figure 1. BDNF discriminated between TRACK-TBI Pilot cases and JHH non-TBI controls with an AUC of 0.94 (95% CI: 0.91-0.97; Fig. 2). BDNF values were lower in TRACK-TBI Pilot cases (prospectively collected samples) than in the JHH or SFGH cohorts (excess clinical samples; $p < 0.001$). BDNF discriminated between JHH non-TBI controls and TRACK-TBI cases classified as mild TBI with an AUC of 0.95 (95% CI: 0.92-0.98).

Association between BDNF and TBI severity

Within the TRACK-TBI Pilot cohort, day-of-injury BDNF values (ng/mL) were higher in mild TBI subjects (8.3; IQR, 5.2-16.5) than in moderate (4.3; IQR, 1.8-10.1) or severe TBI (4.0; IQR, 1.5-13.8; $p = 0.003$). The JHH and SFGH cohorts did not have sufficient moderate and severe TBI patients to assess BDNF variation with TBI severity (Table 1). Among TRACK-TBI Pilot subjects, median day-of-injury BDNF values (ng/mL) were higher in subjects without intracranial abnormality on head CT (8.4; IQR, 5.2-16.6) than in subjects with intracranial abnormality on head CT (4.2; IQR, 1.8-10.1; $p < 0.001$; Fig. 3). BDNF discriminated between subjects with and without intracranial abnormality on head CT with an AUC of 0.67 (95% CI: 0.58-0.75). In the JHH cohort, median BDNF (ng/mL) for normal CT and abnormal head CT were 17.8 (IQR, 12.5-30.8) and 16.2 (IQR, 4.8-23.2), respectively ($p = 0.13$). Whereas in the SFGH cohort, median BDNF (ng/mL) for normal and abnormal head CT were 13.0 (IQR, 9.4-17.1) and 15.1 (IQR, 10.5-21.3), respectively ($p = 0.17$.

Association between BDNF and TBI outcomes

Among the 159 TRACK-TBI Pilot subjects, 94 (59%) had the Rivermead Post-Concussion Questionnaire measured and 111 (69%) had the GOSE score measured at 6 months post-injury. Of those with 6-month outcome measures, 62% (58/94) were determined to have PCS, 70% (78/111) had a GOSE < 8, and 80% (85/106) had either PCS or GOSE < 8. Among the 94 subjects with both PCS and GOSE measures, 51 (54%) had both PCS and GOSE < 8, 21 (22%) had neither PCS nor GOSE < 8, 15 (16%) had GOSE < 8 but no PCS, and seven (7%) had PCS and GOSE $= 8$. Day-of-injury BDNF values (ng/mL) were not significantly different between subjects with PCS (7.2; IQR, 3.0-12.8) and those without PCS (7.1; IQR, 4.0-21.0), or between subjects with GOSE $= 8$ (7.9; IQR, 4.0-23.3) and those with GOSE < 8 (7.1; IQR, 2.8-13.0). The 76 (72.4%) TRACK-TBI subjects who had very low BDNF values (i.e., less than the 1st percentile for non-TBI controls [< 14.2 ng/mL]) had higher odds of incomplete recovery than those without very low BDNF (odds ratio, 4.0; 95% CI: 1.5-11.0). Very low BDNF values were associated with higher odds of incomplete recovery among those with mild TBI (4.9; 95% CI: 1.3-17.9) than those with moderate or severe TBI (2.0; 95% CI: 0.3-12.5).

There was a trend toward higher BDNF values as the time interval between injury and serum sampling for BDNF measurement increased (Fig. 4). The trend was similar among those with

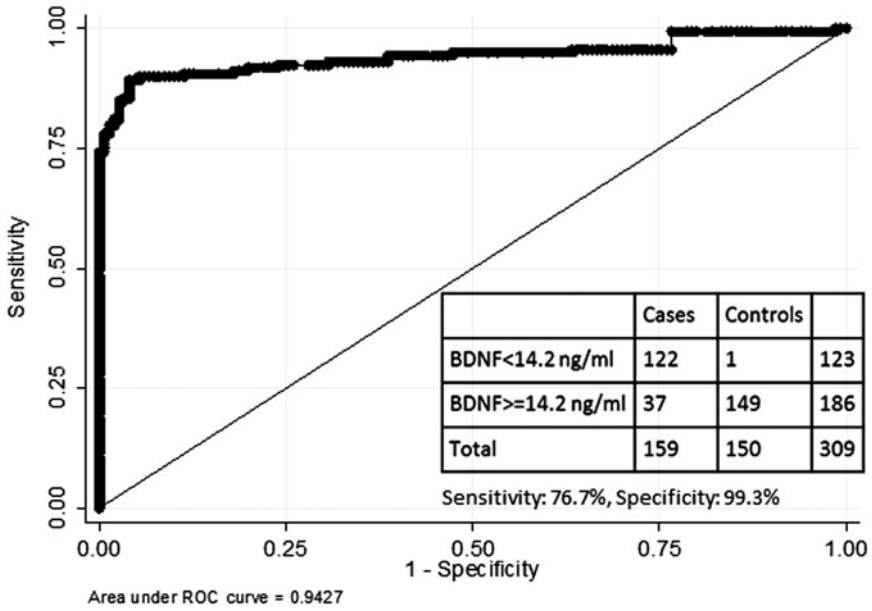

FIG. 2. Receiver operator curve for distinguishing between traumatic brain injury (TBI) cases and controls with brain-derived neurotrophic factor (BDNF). The receiver operator curve for discriminating between Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) Pilot cases and Johns Hopkins Hospital (JHH) controls using BDNF values. The table reports the diagnostic accuracy of using a cut-off value of 14.2 ng/mL for distinguishing between TBI cases and controls.

complete and incomplete recovery. However, this trend did not reach statistical significance ($p=0.10$). Similarly, there was a trend toward lower BDNF values with increasing age (Fig. 5). However, this trend was not statistically significant ($p=0.09$). After adjustment for age and time between injury and serum sampling for BDNF measurement, very low BDNF (<14.2 ng/mL) remained statistically significantly associated with incomplete recovery (odds ratio, 4.16; 95% CI: 1.48-11.70).

Performance of GFAP and UCH-L1, compared with BDNF

A comparison of TRACK-TBI Pilot GFAP and UCH-L1 values with BDNF assayed on the same samples showed that GFAP, BDNF and UCH-L1 discriminated between subjects with traumatic abnormalities on head CT and those without, with AUCs of 0.88 (95% CI: 0.83-0.93) for GFAP, 0.70 (95% CI: 0.62-0.79) for UCH-L1, and 0.67 (95% CI: 0.58-0.75) for BDNF. They also discriminated between subjects with complete recovery from TBI and those without, with AUCs of 0.65 (95% CI: 0.52-0.78) for BDNF, 0.61 (95% CI: 0.49-0.73) for GFAP, and 0.55 (95% CI: 0.43-0.66) for UCH-L1 at 6 months. A comparison of the discriminative abilities of the biomarkers examined is presented in Table 3. There was no minimal correlation between BDNF and GFAP values ($r=-0.11$; $p=0.16$) and between BDNF and UCH-L1 values ($r=0.07$; $p=0.36$), suggesting that they may be associated with different pathways of injury. To determine whether combining biomarkers resulted in improved discrimination of complete versus incomplete recovery, we used combinations of two biomarkers, instead of all three biomarkers, since only 21 subjects had complete recovery (10 events per predictor variable is required for adequate statistical power).[38] Addition of GFAP to BDNF did not improve the discrimination of complete versus incomplete recovery (AUC was 0.66 instead of 0.65; $p=0.76$). Similarly, addition of UCH-L1 to BDNF did not improve the discrimination of complete versus incomplete recovery (AUC was 0.66 instead of 0.65; $p=0.55$).

Predictors of BDNF values in non-TBI control subjects

Among non-TBI controls, after adjustment for age, gender, race, hypertension, diabetes, history of psychiatric illness, and mean arterial pressure, only gender and mean arterial pressure remained independent predictors of BDNF among non-TBI controls (Table 4). Median BDNF levels (ng/mL) were greater in females (69.1; IQR, 41.4-82.4; $n=79$) than in males (52.7; IQR, 38.7-71.8; $n=71$; $p=0.049$). However, there were no gender differences in BDNF levels within the TBI cohorts examined. Among non-TBI controls, BDNF values increased with increasing mean arterial pressure. However, there was no statistically significant association between BDNF and blood pressure within the TBI cohorts examined.

Discussion

We report the diagnostic value of day-of-injury circulating BDNF for TBI, and its ability to be prognostic for identifying subjects likely to have persistent TBI-related sequelae at 6 months. Further, we have determined that BDNF has a higher prognostic value among mild TBI subjects than moderate/severe TBI subjects. The dysregulation of BDNF in TBI has been examined with equivocal findings by a number of studies using animal models of TBI.[10] In the majority of these studies, BDNF mRNA expression was measured in brain tissue, with reports of upregulation of BDNF mRNA in the hippocampus and cerebral cortex.[39–41] However, other studies have suggested reduced secretion of brain BDNF protein after TBI, with subsequent increased secretion following experimental TBI treatment.[42] Few studies have measured circulating BDNF in human TBI subjects. Two small pediatric studies reported no differences in plasma BDNF levels between human

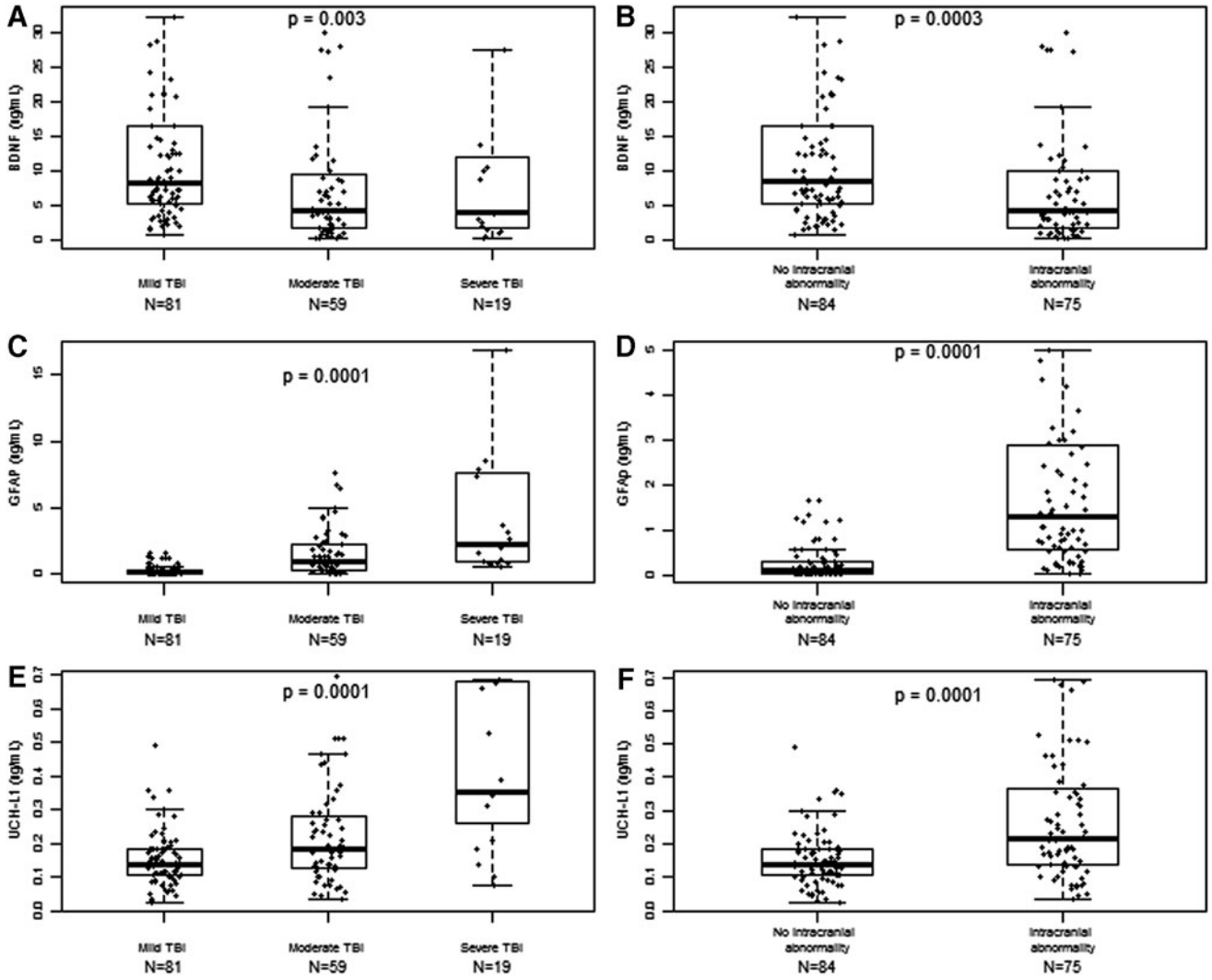

FIG. 3. Association between biomarkers examined and traumatic brain injury (TBI) severity. Presented are the graphical distribution of individual brain-derived neurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase-L1 (UCH-L1) values in Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) Pilot and the corresponding boxplots according to TBI severity, classified as mild, moderate, or severe; and the presence or absence of traumatic intracranial abnormality on head computed tomography (CT) scan: **(A)** depicts BDNF versus TBI severity classified as mild moderate or severe; **(B)** depicts BDNF versus TBI severity classified by CT scan; **(C)** depicts GFAP versus TBI severity classified as mild, moderate, or severe; **(D)** depicts GFAP versus TBI severity classified by head CT scan; **(E)** depicts UCH-L1 versus TBI severity classified as mild, moderate or severe; **(F)** depicts UCH-L1 versus TBI severity classified by head CT scan. Individual values that were extreme outliers are excluded from the graphical presentation.

TBI cases and non-trauma controls.[43,44] However, control subjects in these studies had abnormal neurologic status (obstructive hydrocephalus undergoing elective surgery,[43] and subjects undergoing lumbar puncture for suspected meningitis[44]).

Another study measuring BDNF in Olympic boxers and healthy controls also reported no differences in plasma BDNF.[45] However this study measured BDNF in plasma samples obtained 1–6 days after a bout and the release and clearance kinetics of BDNF in humans is not known. Further, Buonora and colleagues recently reported higher plasma BDNF levels in TBI cases, compared with controls.[46] Our findings and study design are most similar to results reported by Kalish and Phillips.[47] These investigators measured BDNF in serum samples obtained from 30 TBI patients and reported decreasing BDNF with increasing severity of TBI. Our study has demonstrated in three separate TBI cohorts that circulating levels of BDNF are lower in TBI cases, compared with non-trauma controls.

BDNF is limited in its ability to distinguish between TBI subjects with and without intracranial abnormalities. This may be due to the fact that structural proteins (such as GFAP) are more likely to have a strong association with radiographic changes in TBI than secreted proteins. However, secreted proteins may reflect both primary and secondary brain injury and therefore may have a stronger association with long-term outcomes. Our findings demonstrate that BDNF has higher prognostic value in mTBI subjects, compared with moderate or severe TBI patients. Therefore, BDNF holds promise for improving clinical prognostication of outcomes in TBI patients who have no intracranial abnormalities on head CT scans.

BDNF is an appealing candidate biomarker for detecting TBI for numerous reasons. First, our study results demonstrate a very strong

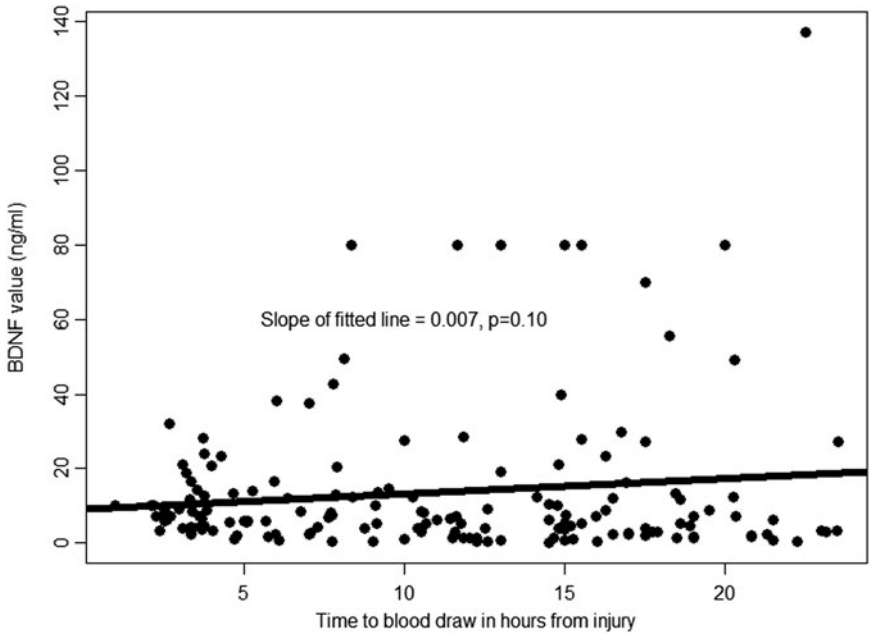

FIG. 4. Association between brain-derived neurotrophic factor (BDNF) and time from injury to blood sampling. This is a scatter plot of the association between day-of-injury BDNF values and time between injury and blood draw (in hours). The line represents the best fitting linear regression line that summarizes this association.

association between BDNF and TBI, yielding excellent discriminative ability of 0.94-0.95 (as measured by the c-statistic). Second, our findings were replicated across three different TBI cohorts. Third, we have demonstrated an association between BDNF and TBI severity and an association between BDNF and TBI outcome. Finally, the association between TBI and BDNF is biologically plausible and has been demonstrated in diverse TBI models including animal models.[10]

BDNF is the most abundantly expressed brain neurotrophin[48] and as a secreted protein, can be readily and reliably measured in serum using well established immuno-assay techniques, identifying it as a non-necrosis brain injury biomarker. This distinguishes BDNF from other protein-based biomarkers that are structural components of neurons and glial cells—for example, GFAP (an astro-glial intermediate filament cytoskeletal protein), S100B (an intracellular calcium binding protein), UCHL1 (a ubiquitin ligase

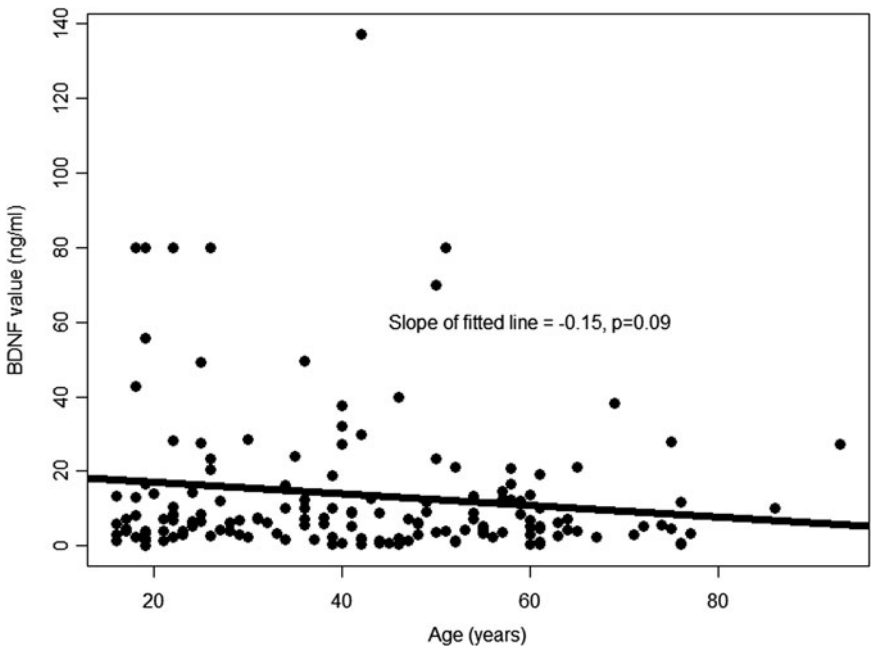

FIG. 5. Association between brain-derived neurotrophic factor (BDNF) and age. This is a scatter plot of the association between day-of-injury BDNF values and age (in years). The line represents the best fitting linear regression line that summarizes this association.

Table 3. Discriminative Ability of Different Biomarkers for Relevant TBI Outcomes as Measured by the Area Under the Receiver Operator Curve (AUC) and the Corresponding 95% Confidence Interval

Outcome	*GFAP*	*UCH-L1*	*BDNF*
GOSE score < 8	0.61 (0.50-0.71)	0.55 (0.44-0.66)	0.56 (0.44-0.68)
Post-concussive syndrome (PCS)	0.56 (0.44-0.68)	0.52 (0.40-0.64)	0.55 (0.43-0.68)
Composite (GOSE score < 8 or PCS)	0.61 (0.49-0.73)	0.55 (0.43-0.66)	0.65 (0.52-0.78)
Intracranial abnormality on head CT	0.88 (0.83-0.93)	0.70 (0.62-0.79)	0.67 (0.58-0.75)

TBI, traumatic brain injury; GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase-L1; BDNF, brain-derived neurotrophic factor; GOSE, Glasgow Outcome Scale Extended; CT, computed tomography.

localized to the neuronal soma), neurofilaments (cytoskeletal components of axons), cleaved tau (intracellular microtubule-associated proteins), and myelin basic proteins (a component of myelin), among others.[49] In order for structural proteins to be found in high abundance in circulation, sufficient cellular necrosis and damage to the BBB is required. However, BDNF does not require cellular necrosis or damage to the BBB to be observed in circulation.[50] Further, this allows BDNF to be more abundant in circulation than structural proteins, increasing assay sensitivity.

The exact mechanisms underlining the dysregulation of BDNF in TBI are not yet well understood. Although some studies implicate BDNF in neuroprotection following injuries,[51,52] other studies suggest it contributes to neurodegenerative events that occur following injury.[53,54] It also has been suggested that BDNF ameliorates the impact of secondary brain damage by modifying BDNF-induced gene expression.[10] Following TBI and acute disconnection of brain circuitry, there is an attempt at reorganization and reconnection of brain circuits. BDNF promotes synaptic plasticity and restoration during the brain circuitry ''reconnection'' phase. We have found that post-TBI BDNF levels behave unlike the majority of candidate biomarkers of TBI. Lower BDNF values are associated with worse prognosis, whereas with other TBI biomarkers, lower values are typically associated with better prognosis,[4] with the exception of microtubule-associated protein 2, a dendritic marker, which has higher values at 6 months after injury in severe TBI subjects with improved outcomes.[3] We postulate that during the acute phase of TBI, the formation of new neuronal circuits might not be advisable, and therefore there may be no need for increased production of neurotrophic factors. However, it is possible that the initial decrease in circulating BDNF during the acute phase of trauma (as seen in our study) is potentially followed by a subsequent increase, especially during the sub-acute/chronic phases of TBI. Understanding the temporal variations in BDNF expression will be an important first step towards further elucidating the biological functions of BDNF in TBI and recovery. It is also possible that since decreased BDNF levels are found in patients with anxiety,[55] major depressive disorder,[56] and schizophrenia,[57] low BDNF values on the day of injury identifies subjects at risk for these conditions (whether previously recognized or otherwise) and predisposes this population to incomplete recovery.

Although circulating BDNF may originate from the hippocampus, cerebral cortex, and basal forebrain,[58] it also may be derived from other cellular sources, including platelets,[59,60] smooth muscle cells,[35,61] and vascular endothelial cells.[62] This supports BDNF's role as a promoter of neuronal growth and survival both in the central and peripheral nervous system. However, it is unclear whether circulating BDNF values measured in this study are representative of central nervous system values. Prior studies suggest that BDNF crosses the BBB bi-directionally.[63] Further, it has been reported that serum and cortical BDNF values are strongly correlated.[64] Irrespective of the exact source(s) of circulating BDNF, our finding that circulating BDNF values are suppressed in TBI and that low BDNF values are associated with poor recovery suggest that BDNF deserves further evaluation as a potential biomarker of TBI and TBI recovery.

BDNF has the potential to become a surrogate marker of successful TBI treatment. In a study examining dietary omega-3 fatty acid supplementation in TBI, rats with decreased brain BDNF following mild fluid percussion injury had normalized brain BDNF levels and improved learning ability following 4 weeks of dietary supplementation with omega-3 fatty acids.[42] Similarly, rats exposed to delayed exercise (2–3 weeks after injury) had increases in

Table 4. Determinants of BDNF in the Control Population ($N = 150$)

	Unadjusted regression co-efficient (95% CI)	*Adjusted regression co-efficient (95% CI)*	*p Value for adjusted regression co-efficient*
Age in years	-0.1 (-0.4 to 0.3)	-0.2 (-0.5 to 0.2)	0.38
Gender			
• Female	Reference	Reference	0.04
• Male	-7.2 (-15.4 to 1.0)	-8.8 (-17.0 to -0.6)	
Race			
• African-American	Reference	Reference	
• Caucasian	-9.6 (-19.8 to 0.7)	-8.8 (-18.8 to 1.3)	0.09
• Other	0.2 (-25.3 to 25.7)	0.6 (-24.8 to 26.0)	0.96
Mean arterial pressure per 10 mm Hg	2.8 (0.7 to 4.9)	3.0 (0.9 to 5.1)	< 0.01
History of hypertension	-0.0 (-9.1 to 9.0)	-0.3 (-9.7 to 9.1)	0.95
History of depression or schizophrenia	-1.2 (-12.7 to 10.2)	-3.3 (-14.6 to 8.0)	0.57

BDNF, brain-derived neurotrophic factor; CI, confidence interval.

BDNF and improved cognitive performance, compared with rats exposed to early (0-6 days) exercise.[65] In our study, low BDNF levels were associated with incomplete recovery at 6 months in individuals with TBI. Further studies are needed to validate this finding and to determine how well longitudinal BDNF values reflect recovery and clinical improvement post-TBI and the BDNF pathway as a therapeutic target.

Decreased circulating BDNF levels have been implicated in other non-TBI conditions including anxiety,[55] major depressive disorder,[56] schizophrenia,[57] and Alzheimer's disease.[66] However, these studies did not account for other potential confounders, such as age and gender. In our study, although control subjects with a history of a psychiatric disorder had lower median BDNF values than those without a history of a psychiatric disorder, this difference was not statistically significant. Additionally, after adjustment for age, gender, race, hypertension, diabetes and mean arterial pressure, history of psychiatric disorder was not an independent predictor of BDNF levels, whereas mean arterial pressure and gender were independent predictors of BDNF in control subjects. However, our findings regarding gender suggest that gender-specific cut-offs may be important in determining the reference values of BDNF. Since BDNF values increase during exercise, it is possible that in the case of sports-related concussions, increases in BDNF from exercise may mask a concussion-related decrease. Additional studies are needed to investigate BDNF levels in sports-related concussions.[67]

Limitations

Our study has a number of limitations. First, storage procedures for serum samples for JHH and SFGH TBI cases and JHH non-trauma controls were different. However, since our findings were reproduced in the TRACK-TBI Pilot cohort, it is unlikely that this discrepancy had an important influence on our study result. Further, BDNF increases with increased duration of storage at room temperature,[25] and that may explain why BDNF values in the JHH and SFGH cohorts are higher than BDNF in TRACK-TBI Pilot.

Additionally, the demographic distribution of our TBI cases was different from that of the non-TBI controls. However, the diagnostic accuracy of BDNF for discriminating between TBI cases and controls did not vary significantly after adjustment for potential confounders. Another major limitation is that the JHH controls had not been exposed to trauma. Since a common clinical challenge is to determine if TBI is present in patients who have been involved in automobile accidents, falls, or blast exposures, an important control group would be individuals exposed to orthopedic or systemic trauma but not head injury. Efforts to collect these ''other injury'' controls are under way.

In our validation cohort, the prevalence of traumatic intracranial abnormalities on head CT scan was much higher (47.2% of TRACK-TBI Pilot cases studied) than that reported in studies that are more representative of the population of ED patients evaluated for TBI.[68,69] Thus, examining the validity of our findings in cohorts that are more representative of ED patients evaluated for TBI will be important.

Conclusion

Serum BDNF discriminates between TBI cases and non-trauma controls with excellent diagnostic accuracy. Additionally, lower BDNF values are associated with incomplete recovery after TBI, and may be especially useful in identifying mild TBI patients who are likely to remain symptomatic at 6 months after injury.

Author Disclosure Statement

Under a licensing agreement between ImmunArray and the Johns Hopkins University, Drs. Everett, Korley, and Van Eyk are entitled to royalties on an invention described in this article.

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Outcome Prediction after Mild and Complicated Mild Traumatic Brain Injury: External Validation of Existing Models and Identification of New Predictors Using the TRACK-TBI Pilot Study

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Abstract

Although the majority of patients with mild traumatic brain injury (mTBI) recover completely, some still suffer from disabling ailments at 3 or 6 months. We validated existing prognostic models for mTBI and explored predictors of poor outcome after mTBI. We selected patients with mTBI from TRACK-TBI Pilot, an unselected observational cohort of TBI patients from three centers in the United States. We validated two prognostic models for the Glasgow Outcome Scale Extended (GOS-E) at 6 months after injury. One model was based on the CRASH study data and another from Nijmegen, The Netherlands. Possible predictors of 3- and 6-month GOS-E were analyzed with univariate and multi-variable proportional odds regression models. Of the 386 of 485 patients included in the study (median age, 44 years; interquartile range, 27–58), 75% ($n = 290$) presented with a Glasgow Coma Score (GCS) of 15. In this mTBI population, both previously developed models had a poor performance (area under the receiver operating characteristic curve, 0.49–0.56). In multivariable analyses, the strongest predictors of lower 3- and 6-month GOS-E were older age, pre-existing psychiatric conditions, and lower education. Injury caused by assault, extracranial injuries, and lower GCS were also predictive of lower GOS-E. Existing models for mTBI performed unsatisfactorily. Our study shows that, for mTBI, different predictors are relevant as for moderate and severe TBI. These include age, pre-existing psychiatric conditions, and lower education. Development of a valid prediction model for mTBI patients requires further research efforts.

Key words: GOS-E; prognostic models; TBI; validation

Introduction

Traumatic brain injury (TBI) is among the leading causes of death and disability. In the United States, at least 1.7 million patients a year seek some form of medical treatment.[1] TBI exacts significant health, social, and economic hardships on patients, their families, and health systems.[2,3] Approximately 70–90% of all TBIs are categorized as mild (mTBI), that is, presenting with a Glasgow Coma Scale (GCS) score of 13–15 after nonpenetrating head trauma. Although most mTBI patients will recover without residual impairments, persistent sequelae remain in a subgroup of 5–15%.[4] These complaints may include physical symptoms, behavioral disturbances,

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and cognitive dysfunction, any of which may interfere with return to work or resumption of social activities. Prognostic analyses are essential to identify patients at increased risk of developing residual sequelae and for leveraging resources to follow a more risk-prone subgroup. Closer observation and early intervention as part of clinical practice may alleviate the psychological burden of injury on these patients, as well as the related economic burden on society.

The heterogeneity in case definition of mTBI, the variety of outcome measures, and the variability in time elapsed for scoring both predictors and outcome render interpretation and comparison of results from mTBI prognostic studies difficult. Further, most studies only report on the association between predictors and outcome in univariate analyses.[5,6]

To our knowledge, only two studies have combined predictors and developed a prediction model specifically for mTBI.[7,8] One other model (Corticosteroid Randomization After Significant Head Injury; CRASH) was developed on patients with GCS 3–14 and thus captured a segment of the mTBI population, but not patients with GCS 15.[9,10] Further, none of the models have been externally validated in mTBI. Before a prognostic model can reliably be applied to clinical practice, external validation is required to determine generalizability. In this study, we aimed to evaluate the performance of existing mTBI prognostic models using a recent, prospective, unselected population of mTBI patients enrolled across three level 1 trauma centers in the United States and explore relevant predictors of poor outcome after mTBI.

Methods

Patient population

The study population consisted of patients included in the **T**ransforming **R**esearch **a**nd **C**linical **K**nowledge in TBI (TRACK-TBI) Pilot study.[11] In this study TBI patients age >16 years were enrolled upon arrival in the emergency departments (EDs) at San Francisco General Hospital (University of California San Francisco; UCSF), University of Pittsburgh Medical Center, and University Medical Center Brackenridge. All participants or their legally authorized representatives gave written informed consent. At follow-up outcome assessments, participants previously consented by legally authorized representative, if neurologically improved and capable, were consented for continuation in the study.

Inclusion criteria were presentation to study hospital within 24 h of injury and history of trauma to the head sufficient to triage to noncontrast head computed tomography (CT) using the American College of Emergency Physicians/Centers for Disease Control evidence-based joint practice guidelines.[12] We selected patients with mTBI and available 3- or 6-month outcome. All study protocols were approved by the institutional review boards at each participating level 1 trauma center.

Measures

Details on loss of consciousness, amnesia, and source of trauma were recorded upon admission and informed consent was obtained. GCS score was assessed by a neurosurgeon at admission.[13] Trained study personnel in the ED obtained demographic data, patient history, and clinical information from the patient. All patients underwent CT imaging at the time of initial presentation to the ED. Each patient's head CT was characterized using the National Institutes of Health/National Institute of Neurological Disorders and Stroke TBI Common Data Elements (TBI-CDEs).[14–16] Clinical brain CTs were transmitted to a radiology picture-archiving and communications system with software that allow controlled remote access for multiple users at study sites. To comply with the Health Insurance Portability and Accountability Act of 1996, the UCSF Quantitative Image Processing Center built a multiplatform tool that completely anonymized CT studies during the transmission process. Each CT was then reviewed by a single board-certified neuroradiologist blinded to demographic, socioeconomic, and clinical data, except gender and age, and scored on 26 of the 93 CDEs developed by the TBI-CDE neuroimaging working group.[17,18]

Outcome

The outcomes for this study were the Glasgow Outcome Scale Extended (GOS-E) at 3 and 6 months after injury.[19] The GOS-E provides eight categories of outcome: dead; vegetative state; lower severe disability; upper severe disability; lower moderate disability; upper moderate disability; lower good recovery; and upper good recovery. Ratings are based on patient consciousness, independence, ability to work, social and leisure activities, social relationships, and other sequelae of TBI. Upper good recovery (GOS-E score of 8) indicates return to preinjury baseline with no residual effects of the TBI.

Prediction models

Our literature search identified three prediction models that were developed (partly) on mTBI patients.[7–9] We could not validate the Stuhlemeijer and colleagues model because not all of the former's predictors were available in our data set.[7] We thus undertook to validate the Nijmegen and CRASH models.[9] The characteristics of the model are described in Table 1.

The Nijmegen model was built specifically for mTBI, with 6-month GOS-E <7 as the endpoint. Multivariable analysis of 1069 patients with GOS-E yielded age, Abbreviated Injury Score for head (AISh), Injury Severity Score (ISS) without head, and alcohol intoxication as significant predictors in the clinical model and number of hemorrhagic contusions and facial fractures as predictors of unfavorable outcome in the CT model and age, ISS without head, number of hemorrhagic contusions, and alcohol intoxication in the combined model.[8]

The Medical Research Council CRASH trial built and externally validated two prognostic models in mild, moderate, and severe TBI.[9] A basic model included age, GCS, pupillary reactivity, and presence of extracranial injury. In a CT model, additionally included were petechial hemorrhage, obliteration of third ventricle and cisterns, subarachnoid hemorrhage (SAH), mid-line shift, and nonevacuated hematoma emerged as predictors for mortality at 14 days and unfavorable outcome on the GOS (<4) at 6 months postinjury.[9] In this study, we only validated the models for 6-month unfavorable outcome. We note that the CRASH model excluded patients with GCS 15, a score that represents a majority of this subpopulation.

Statistical analysis

If patients had a missing outcome at 6 months, but an observed outcome at 3 months, the 3-month value was extrapolated to 6 months. Similarly, 6-month outcomes were interpolated when 3-month outcome was missing. Patients with missing outcome at both time points were excluded. Missing values in predictors were statistically imputed using single imputation with the AregImpute function in R statistical software (version 2.14; R Foundation for Statistical Computing, Vienna, Austria).

Patients' baseline characteristics were described by median and interquartile range (IQR) for continuous variables and frequencies and percentages for categorical variables. These descriptive statistics were reported on the nonimputed data.

The prediction models were applied to the patients in the validation set, that is, a predicted probability of unfavorable outcome was calculated for each patient using the CRASH and Nijmegen models. Accordingly, the external validity of the models was assessed by studying calibration and discrimination. Calibration refers to the agreement between observed and predicted outcomes. The

Table 1. Characteristics of the Validated Models

Model	*Development population (*n*)*	*Predictors*	*Outcome*
Nijmegen	GCS 13–15 ($n=1069$)		6-month GOS-E < 7
Clinical model		-Age -AIS head -ISS without head -Alcohol intoxication	
CT model		-Number of hemorrhagic contusions -Facial fractures	
Combined model		-Age -ISS without head -Number of hemorrhagic contusions -Alcohol intoxication	
CRASH	GCS 3–14 ($n=10{,}008$)		6-month GOS < 4
Basic model		-Age -GCS -Pupillary reactivity -Extracranial injury	
CT model		Basic model plus -Petechial hemorrhage -Obliteration of third ventricle and cisterns -Subarachnoid hemorrhage -Mid-line shift -Nonevacuated hematoma	

CT, computed tomography; CRASH, Corticosteroid Randomization After Significant Head Injury; GCS, Glasgow Coma Scale; AIS, Abbreviated Injury Score; ISS, Injury Severity Score; GOS-E, Glasgow Outcome Score Extended.

extent of over- or underestimation, relative to the observed and predicted rate, was explored graphically using validation plots.[20] We assessed calibration-in-the-large by fitting a logistic regression model with the logit of model predictions as an offset variable. The intercept indicates whether predictions are systematically too low or high and should ideally be zero. The calibration slope reflects the average effects of the predictors in the model and was estimated in a logistic regression model with the logit of the model predictions as the only predictor. For a perfect model, the slope is equal to 1. The area under the receiver operating characteristic curve (AUROC) was used to quantify the ability of the model to discriminate between patients who died versus survived. Because the development of the CRASH model did not include patients with GCS 15, we validated it both on patients with GCS 13–14 and on our total study population.

To further explore relevant predictors of 3- and 6-month GOS-E, we selected 21 possible predictors from the literature and based on clinical knowledge. These were analyzed in univariate and multivariable proportional odds regression models with 3- and 6-month GOS-E as ordinal outcomes. This means that the full range of the GOS-E is considered instead of dichotomizing at a fixed point (e.g., favorable vs. unfavorable outcome). Simulation studies have shown that ordinal analysis is more efficient than dichotomization, also when the proportional odds assumption is violated. Each predictor was tested in the univariate models, and those with a p value of 0.30 in both the 3- and 6-month model were selected for inclusion in the multi-variable models. The liberal p value was motivated by the fact that we performed an exploratory analysis in a relatively small sample size and did not want to exclude possible predictors.

All analyses were performed with R statistical software (version 2.14; R Foundation for Statistical Computing).

Results

Patient population

TRACK-TBI Pilot enrolled 485 patients with mTBI, including 480 with nonpenetrating injury who were eligible for our study. Patients with penetrating brain injury ($n=5$) or missing outcome at both 3 and 6 months after injury ($n=94$) were excluded. A total of 386 patients were included in our analysis. The median age of our population was 44 years (IQR, 27–58). The majority ($n=271$; 70%) was male. Most patients ($n=290$; 75%) presented with a GCS of 15 and two reactive pupils. Most patients were injured in a motor vehicle traffic accident ($n=179$; 47%). Almost one third ($n=118$; 31%) of the patients had self-reported psychiatric (mental health) history, which was obtained at the time of injury through patient interview using a checklist of common psychiatric conditions as defined by the TBI CDE V1.0 (e.g., anxiety, depression, sleep disorders, post-traumatic stress, bipolar disorder, schizophrenia, and others). Patients need not have been formally diagnosed with a mental health disturbance; however, to qualify as ''positive'' for psychiatric history, the patient must deem the condition to be significantly disturbing for their baseline quality of life. More then half ($n=198$; 53%) of the patients reported history of previous TBI as defined by external force injury to the head. Over half of the patients ($n=232$; 60%) had no visible CT pathology (Marshall's CT classification I).[21] The most common pathologies observed on CT were contusions (61; 16%), SAH (103; 27%), and facial fractures (53; 14%). Most baseline variables had very few missing values (< 2%), but the AISh, ISS, and extracranial injury had almost 40% missing values. Alcohol intoxication, as measured by blood alcohol levels, was missing in almost 60% of cases (Table 2).

At 3 months after injury, 116 (24%) were lost to follow-up. Of those with observed outcomes, 33% ($n=121$) completely recovered (GOS-E, 8) and 32% ($n=118$) had some remaining symptoms (GOS-E, 7). Of the remaining one third of the sample 2% ($n=6$) died, 4% ($n=15$) were severely disabled (GOS-E, 3–4), and 28% ($n=104$) were moderately disabled (GOS-E, 5–6; Table 3).

After 6 months, an additional 181 (38%) patients were lost to follow-up. Of those with observed outcome, 34% ($n=102$) made a complete recovery (GOS-E, 8) at 6 months and 30% ($n=89$) had

Table 2. Patient Characteristics ($N = 386$[a])

Characteristic	*Missing*	No. (%)
Age (median, IQR)	0	44 (27–58)
Male gender	0	271 (70)
Cause	4	
Road traffic accident		179 (47)
Fall		133 (35)
Assault		54 (14)
Struck by/struck against person or object		14 (6)
Other		2 (1)
GCS	0	
15		290 (75)
14		81 (21)
13		15 (4)
Pupil reactivity	61	
Both reactive		319 (98)
One reactive		5 (2)
None reactive		1 (0)
Psychiatric medical history	0	118 (31)
Hypoxia	2	23 (6)
Hypotension	1	13 (3)
Previous TBI (with and without hospital admission)	11	198 (53)
Education	12	
Low		37 (10)
Middle		202 (54)
High		135 (36)
Alcohol intoxication	228	52 (33)
ISS (median, IQR)	152	16 (10–18)
AIS head	152	
0		34 (15)
1		6 (3)
2		27 (12)
3		70 (30)
4		83 (35)
5		14 (6)
Extracranial injury	152	53 (23)
Marshall CT	0	
1		232 (60)
2		134 (35)
3		9 (2)
4		4 (1)
5		5 (1)
6		2 (1)
Facial fracture	0	53 (14)
EDH	0	12 (3)
tSAH	1	103 (27)
Mid-line shift	1	10 (3)
Third ventricle obliteration	2	11 (3)
Contusions	1	61 (16)
Petechial hemorrhage	1	3 (1)

[a]Of 485 patients, 5 were excluded because they had penetrating injury and 94 had missing outcome, leaving 386 for inclusion.

IQR, interquartile range; GCS, Glasgow Coma Scale; TBI, traumatic brain injury; ISS, Injury Severity Score; AIS, Abbreviated Injury Score; CT, computed tomography; EDH, extradural haematoma; tSAH, traumatic subarachnoid hemorrhage.

some remaining symptoms (GOS-E, 7). Three percent ($n = 9$) had died, 3% ($n = 9$) were severely disabled (GOS-E, 3–4), and 30% ($n = 90$) were moderately disabled (GOS-E, 5–6).

Between 3 and 6 months after injury, 3 patients died and another 65 deteriorated, based on worsening GOS-E. Conversely, 66 patients showed improved GOS-E scores between 3 and 6 months. The 94 patients with missing outcome at both time points were excluded from this analysis.

Model validation

The Nijmegen models performed poorly in the external validation, with AUROCs of 0.52 (95% confidence interval [CI], 0.49–0.56; clinical model), 0.55 (95% CI, 0.49–0.55; CT model), and 0.56 (95% CI, 0.49–0.56; combined model) (Fig. 1). The CRASH models performed poorly in the total mTBI population, including GCS 15 (AUROC basic model, 0.49; 95% CI, 0.43–0.70; AUROC CT model, 0.49; 95% CI, 0.42–0.66) (Fig. 2). However, performance was very well with AUROCs of 0.90 (95% CI, 0.82–0.97; basic model) and 0.91 (95% CI, 0.85–0.98; CT model) (Fig. 3) in the population they were developed on. The proportion of unfavorable outcome in TRACK-TBI Pilot was overestimated by most models. For example, the predicted proportion of patients with unfavorable outcome by the CRASH CT model was 12%; however, the actual observation of unfavorable outcome at 6 months was 8%.

Predictors

In univariate analyses (Table 4), we identified a large number of characteristics as potential predictors of outcome both 3- and 6-month GOS-E: age; cause of injury; GCS; pupil reactivity; psychiatric medical history; hypoxia; hypotension; education; ISS; extracranial injury; SAH; mid-line shift; and third ventricle obliteration and contusions (all $p < 0.30$ for both 3- and 6-month GOS-E; Table 4). Some predictors had a different effect on 3-versus 6-month outcome. A GCS of 13 or 14 was a strong predictor for a lower 6-month GOS-E (odds ratio [OR] = 0.3; $p = 0.015$), but less predictive for lower 3-month GOS-E (OR = 0.5–0.6; $p = 0.299$). In contrast, the CT characteristics were more predictive of 3-month outcome, compared with 6-month outcome (e.g., SAH: 3-month OR = 2.2, $p < 0.001$; 6-month OR = 1.3, $p = 0.224$).

In multivariable analyses (Table 5), the strongest predictors of both lower 3- and 6-month GOS-E were older age (OR, 1.2; $p < 0.001$), history of psychiatric conditions (OR = 2.2–2.4; $p < 0.001$), and lower education (OR, 0.4–0.8; $p < 0.05$; Table 4). Injury caused by assault and extracranial injury were important predictors of poorer outcome at both time points ($p = 0.05$–0.1). Finally, a lower GCS was predictive of lower 6-month GOS-E (OR, 0.3–0.4; $p = 0.039$).

Discussion

In this study, we externally validated two prognostic models for prediction of outcome after mTBI. We found that both models performed unsatisfactorily in our validation data set. In exploratory analyses, we identified older age, pre-existing psychiatric conditions, lower education, injury caused by assault and extracranial injury, and lower GCS as predictors of 3- and 6-month GOS-E.

Study population

We included only patients with a so-called mTBI, as defined by a GCS 13–15. However, the population did contain some patients

Table 3. Outcome[a]

3-month GOS-E / *6-month GOS-E*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*Unknown*	*Total (%)*
1	6	0	1	0	1	0	1	0	0	9 (3[b])
2	0	0	0	0	0	0	0	0	0	0 (0[b])
3	0	0	2	1	1	0	0	0	1	5 (2[b])
4	0	0	2	1	0	0	1	0	0	4 (1[b])
5	0	0	1	0	14	10	6	4	3	38 (13[b])
6	0	0	0	3	9	13	21	3	3	52 (17[b])
7	0	0	0	1	5	14	43	18	8	89 (30[b])
8	0	0	0	0	2	7	22	64	7	102 (34[b])
Unknown	0	0	0	3	9	19	24	32	94	181 (38[c])
Total (%)	6 (2[b])	0 (0[b])	6 (2[b])	9 (2[b])	41 (11[b])	63 (17[b])	118 (32[b])	121 (33[b])	116 (24[c])	480

[a]$n = 480$.
[b]Percentage of patients with observed outcome.
[c]Percentage of all patients.
GOS-E, Glasgow Outcome Score Extended.

Table 4. Univariate Predictors of 3- and 6-Month GOS-E[a]

Predictors	*Common OR (95% CI) (3 months)*	p *value*	*Common OR (95% CI) (6 months)*	p *value*
Age (per 10 years)	1.2 (1.1–1.3)	< 0.001	1.2 (1.1–1.3)	0.002
Male gender	0.9 (0.6–1.4)	0.678	0.8 (0.6–1.2)	0.316
Cause		0.021		< 0.001
MV	Ref		Ref	
Fall	1.4 (0.9–2.1)		1.6 (1.1–2.4)	
Assault	2.2 (1.3–3.6)		2.6 (1.5–4.5)	
Struck by/strike against	1.3 (0.5–3.4)		0.6 (0.2–1.7)	
GCS		0.299		0.015
13	Ref		Ref	
14	0.6 (0.3–1.6)		0.3 (0.1–1.0)	
15	0.5 (0.2–1.3)		0.3 (0.3–0.7)	
No or one pupil reactive	2.4 (0.6–9.6)	0.205	3.8 (1.1–13.5)	0.039
Psychiatric medical history	2.2 (1.5–3.3)	< 0.001	2.9 (1.9–4.2)	< 0.001
Hypoxia	2.8 (1.3–5.9)	0.009	2.7 (1.2–6.1)	0.018
Hypotension	1.8 (0.7–4.8)	0.206	2.2 (0.8–5.8)	0.112
Education		0.050		0.012
Low	Ref		Ref	
Middle	1.0 (0.5–1.9)		0.7 (0.4–1.4)	
High	0.6 (0.3–1.1)		0.4 (0.2–0.8)	
Alcohol intoxication	0.9 (0.6–1.3)	0.565	1.2 (0.8–1.7)	0.463
ISS	1.03 (1.01–1.06)	0.026	1.02 (0.99–1.04)	0.156
AIS head	1.2 (1.0–1.3)	0.017	1.03 (0.90–1.12)	0.701
Extracranial injury	1.7 (1.1–2.7)	0.012	1.6 (1.0–2.4)	0.044
Marshall's CT		0.002		0.836
1	Ref		Ref	
2	1.9 (1.3–2.8)		1.0 (0.8–1.5)	
3–4	2.9 (1.2–7.6)		1.7 (0.7–4.1)	
5–6	15.5 (3.2–76.2)		8.5 (1.8–40.8)	
Facial fracture	1.4 (0.9–2.4)	0.147	1.3 (0.8–2.3)	0.307
EDH	1.0 (0.4–2.6)	0.986	0.3 (0.1–0.9)	0.033
tSAH	2.2 (1.5–3.3)	< 0.001	1.3 (0.9–1.9)	0.224
Midline shift	7.8 (2.2–27.6)	0.013	3.2 (0.9–11.6)	0.070
Third ventricle obliteration	8.2 (2.6–26.4)	< 0.001	3.2 (1.0–10.3)	0.050
Contusions	1.9 (1.2–3.1)	0.008	1.4 (0.9–2.3)	0.171
Petechial hemorrhage	2.0 (0.3–12.7)	0.473	0.5 (0.1–3.5)	0.527

[a]$n = 386$.
GOS-E, Glasgow Outcome Score Extended; MV, motor vehicle; GCS, Glasgow Coma Scale; ISS, Injury Severity Score; AIS, Abbreviated Injury Score; CT, computed tomography; EDH, extradural haematoma; tSAH, traumatic subarachnoid hemorrhage; OR, odds ratio, CI, confidence interval; Ref, reference.

Table 5. Multivariable Predictors of 3- and 6-Month Ordinal GOS-E

Predictor	*Common OR (95% CI) (3 months)*	p *value*	*Common OR (95% CI) (6 months)*	p *value*
Age (per 10 years)	1.2 (1.1–1.4)	< 0.001	1.2 (1.1–1.4)	< 0.001
Cause		0.103		0.039
MV	Ref		Ref	
Fall	0.9 (0.6–1.4)		1.0 (0.6–1.6)	
Assault	1.9 (1.1–3.4)		2.0 (1.1–3.6)	
Struck by/strike against	1.1 (0.4–3.4)		0.5 (0.2–1.4)	
GCS		0.481		0.061
13	Ref		Ref	
14	0.8 (0.3–2.3)		0.4 (0.1–1.2)	
15	0.6 (0.2–1.7)		0.3 (0.1–0.9)	
No or one pupil reactive	1.0 (0.2–4.4)	0.974	2.1 (0.6–7.5)	0.253
Psychiatric medical history	2.2 (1.4–3.2)	< 0.001	2.4 (1.6–3.7)	< 0.001
Hypoxia	2.0 (0.9–4.4)	0.101	1.8 (0.7–4.2)	0.193
Hypotension	1.4 (0.5–3.6)	0.507	1.6 (0.6–4.1)	0.369
Education		0.032		0.016
Low	Ref		Ref	
Middle	0.8 (0.4–1.6)		0.7 (0.4–1.4)	
High	0.5 (0.2–1.0)		0.4 (0.2–0.9)	
ISS per point	1.02 (0.99–1.04)	0.250	1.00 (0.98–1.03)	0.759
Extracranial injury	1.7 (1.0–2.7)	0.045	1.5 (0.9–2.4)	0.105
tSAH	1.6 (0.9–2.9)	0.095	0.9 (0.5–1.5)	0.579
Mid-line shift	1.6 (0.3–8.6)	0.594	0.8 (0.1–5.2)	0.844
Contusion	1.3 (0.7–2.6)	0.404	1.6 (0.8–3.1)	0.176
Third ventricle obliteration	4.1 (0.8–20.6)	0.084	3.4 (0.6–20.2)	0.181

AUROC 3-month model = 0.68; AUROC 6-month model = 0.69.

GOS-E, Glasgow Outcome Score Extended; MV, motor vehicle; GCS, Glasgow Coma Scale; ISS, Injury Severity Score; tSAH, traumatic subarachnoid hemorrhage; OR, odds ratio, CI, confidence interval; Ref, reference.

with one or two unreactive pupils, an AISh of 4 or 5, or a Marshall's CT classification of 5 or 6, characteristics that indicate a more severe head injury. This illustrates the limitations of a unidimensional approach to classification of TBI. More than half of the patients reported a previous head injury. This might be an overestimation given that it was self-reported.

Outcome

Our findings that one third of the patients made a complete recovery (GOS-E, 8), one third had some minor remaining symptoms (GOS-E, 7), and the final one third had significant disabling complaints at 3 and even 6 months are consistent with previous research.[7] Although our study population might include somewhat more severe patients than the general population as a result of the case mix at our level 1 trauma enrollment centers, these results illustrate that the consequences of mTBI should not be underestimated. The overall outcome distribution was similar at 3 and 6 months, but there were some patients who died between 3 and 6 months and some that deteriorated. Unfortunately, we were unable to trace whether those that deteriorated did so as a result of the initial head injury or from other events. The lost to follow-up percentage increased to 38% at 6 months. This lost to follow-up percentage is similar to, or better than, other TBI studies.[22–24] However, higher follow-up rates are generally achieved in randomized, controlled trials. TBI patients are a difficult group to follow, and researchers should recognize the fact that it requires substantial resources to achieve acceptable follow-up rates in TBI studies.

Approximately half of the patients (94 of 181) who were lost to follow-up at 6 months also did not have a 3-month outcome. Of the patients with observed outcome at 3 months, the majority (56 of 87) had a GOS-E of 7 or 8. This is consistent with previous findings that willingness to participate in research is less in those who fully recover and may result in an overestimation of the rate of unfavorable outcome.[25] Given that it is unlikely that predictors have differential relative effects in patients with more-favorable outcome, we do not expect the results of the prognostic analyses to be affected by the missing outcomes.

Models

With AUROCs of 0.52–0.56, the Nijmegen model's ability to discriminate between patients with favorable and unfavorable outcome was hardly better than chance (AUROC = 0.5). The reason for this poor performance is likely to be related to the original modeling strategy used in this study. Their development sample included 1069 patients, of which 257 had unfavorable outcome. In this sample, 33 possible predictors were tested, corresponding to one predictor for seven outcome events. A rule of thumb in prognostic modeling is that at least 10–20 outcome events are required to test one predictor. Testing too many predictors for the sample size may result in models that are overfitted, resulting in a good apparent performance in the development data, but poor performance at external validation. The amount of overfitting can be assessed and quantified with internal validation (e.g., in a bootstrap procedure), but this was not done by Jacobs and colleagues. The

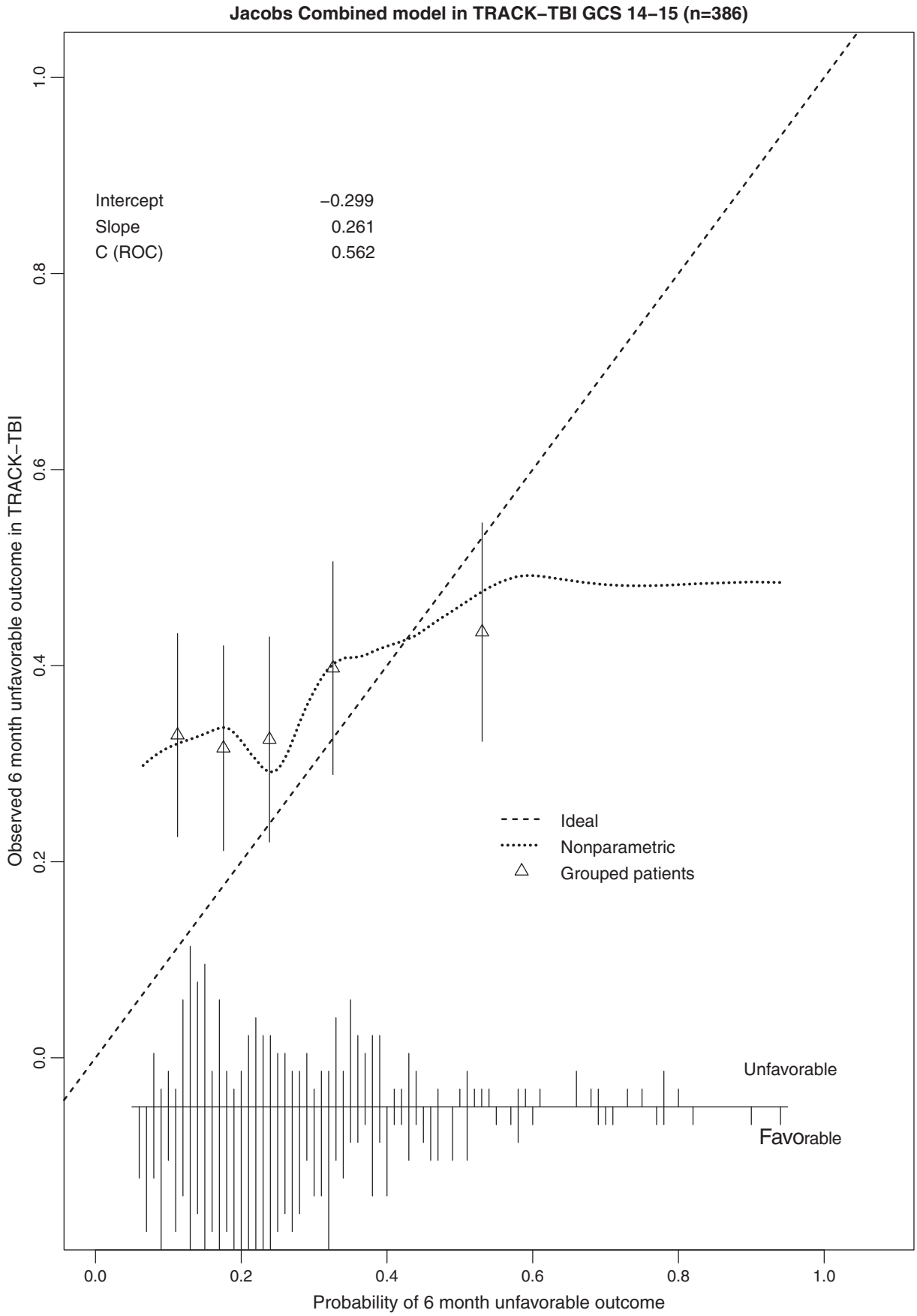

FIG. 1. Calibration plot Jacobs combined model. x-axis shows predicted probabilities by the model in quintiles of patients (triangles with horizontal lines as 95% confidence intervals); y-axis shows observed probabilities. Dotted diagonal represents perfect predictions. Spikes along the x-axis are numbers of patients with favorable and unfavorable observed outcomes. ROC, receiver operating characteristic.

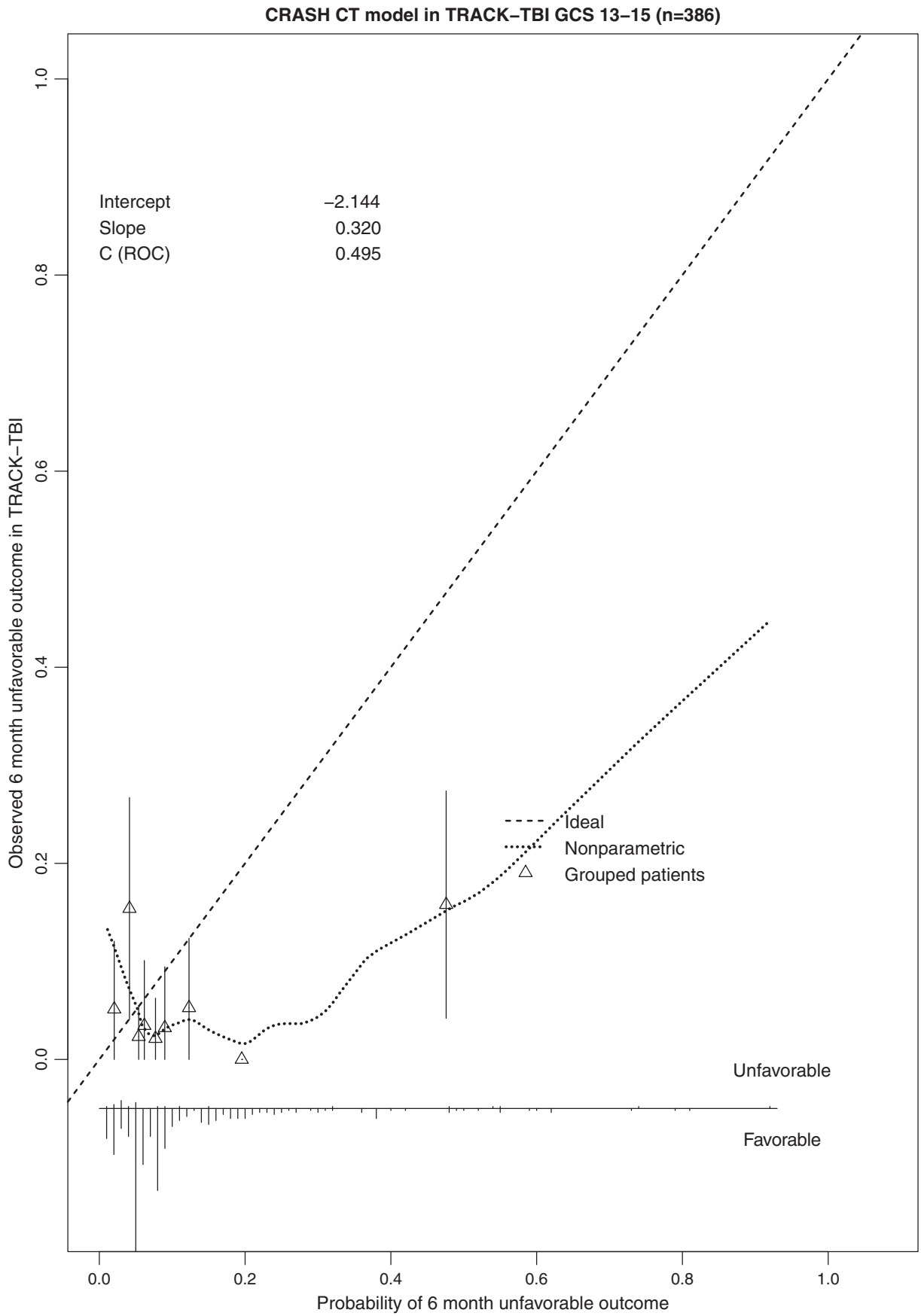

FIG. 2. Calibration plot CRASH computed tomography model. x-axis shows predicted probabilities by the model in quintiles of patients (triangles with horizontal lines as 95% confidence intervals); y-axis shows observed probabilities. Dotted diagonal represents perfect predictions. Spikes along the x-axis are numbers of patients with favorable and unfavorable observed outcomes. ROC, receiver operating characteristic.

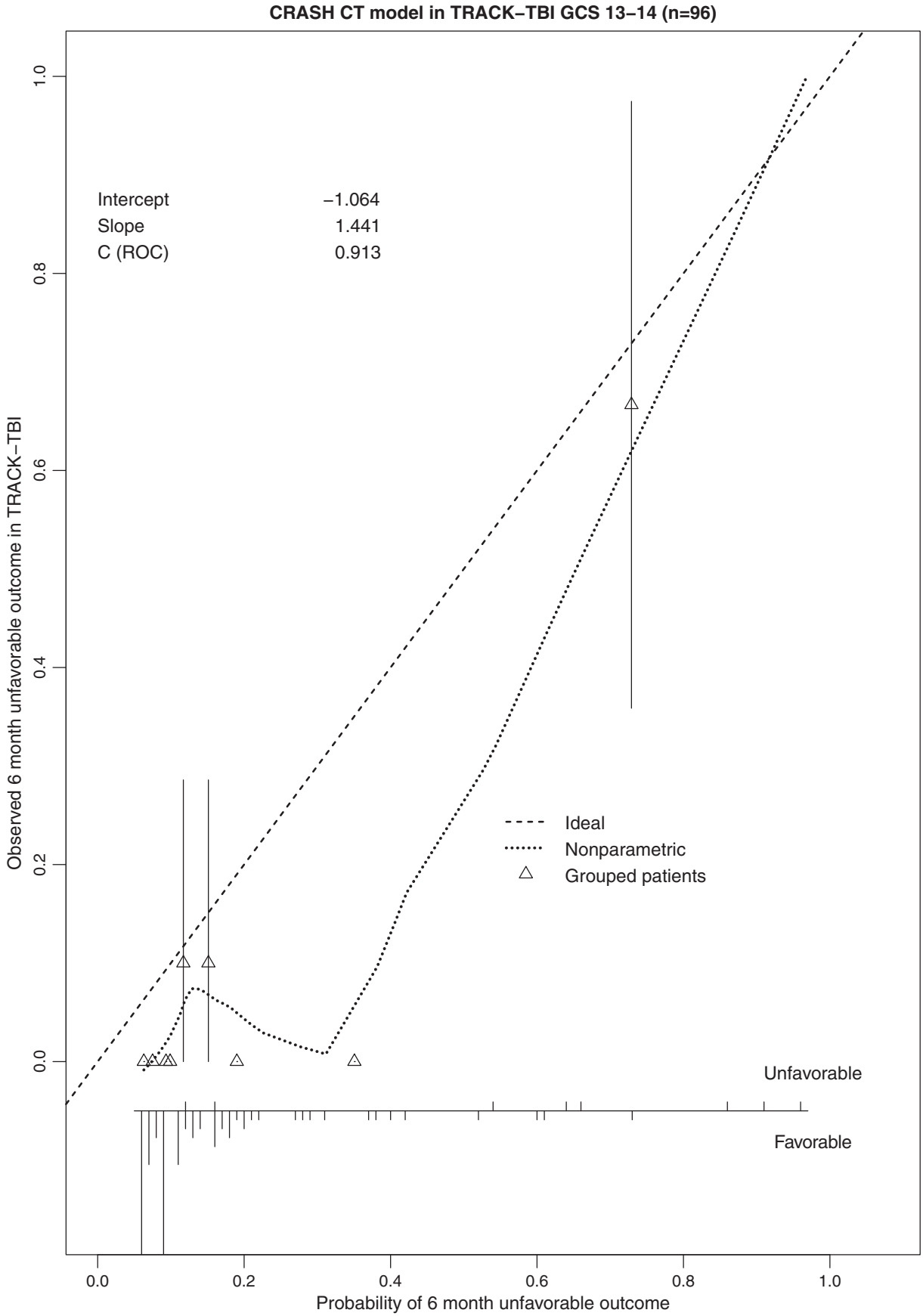

FIG. 3. Calibration plot CRASH computed tomography model (original population). x-axis shows predicted probabilities by the model in quintiles of patients (triangles with horizontal lines as 95% confidence intervals); y-axis shows observed probabilities. Dotted diagonal represents perfect predictions. Spikes along the x-axis are numbers of patients with favorable and unfavorable observed outcomes. ROC, receiver operating characteristic.

difference between the discriminative ability in the development data (AUROCs, 0.57–0.71) and in the validation data likely indicate that the Jacobs model is overfitted, but may also be attributed to true differences in prognostic relations.

The CRASH models discriminated equally poor in the total mTBI population, with AUROCs of 0.49–0.50. However, the CRASH models were not developed for patients with a GCS of 15, which was the majority of our sample. When patients with GCS 15 were excluded, the CRASH models discriminated well. In contrast to the Nijmegen models, the CRASH models were developed by testing 14 predictors in 3556 outcome events and were internally and externally validated in moderate and severe TBI.[26] It should be noted that the outcome predicted by the CRASH models was GOS < 4, whereas the Nijmegen model predicts GOS-E < 7. Possibly, it is easier to discriminate between patients above or below a cutoff in the middle of the GOS-E, compared with a cutoff at the higher end. This is supported by the finding that our ordinal multivariable models had AUROCs of 0.68–0.69, representing the discriminative ability over the complete GOS-E. When the models were refitted with CRASH outcome GOS < 4, the AUCs increased to 0.86. In all, the validation of these previously developed models supports the need for further research to develop valid prognostic models for mTBI patients.

Predictors of unfavorable outcome

Age, pre-existing psychiatric conditions, and lower education were the strongest predictors for both 3- and 6-month GOS-E in our data. Older age is a recognized predictor of poorer outcome in many diseases, including TBI, and our finding is consistent with the literature.[27] Pre-existing psychiatric conditions are less often studied, but also have been found to predict unfavorable outcome.[28] While speculative, it is possible that individuals with a pre-existing mental health condition may have less reserve to overcome the additional strain of an mTBI. Alternatively, symptoms that relate primarily to this comorbidity may falsely be attributed to the head injury.[29] More highly educated patients may have more-adaptive coping skills that allow them to return to their previous levels of functioning.[7]

Additional strong predictors of lower 6-month GOS-E were injury caused by assault, extracranial injury, and lower GCS. GCS is an indication of more-severe injury resulting in less favorable outcome. Violence as a cause of injury has been previously described as a predictor of fatigue after mTBI. The researchers suggested that post-traumatic stress might play a role in this relation.[28] Extracranial injury may result in disability independent of the head injury and has been described as a predictor of poor outcome before, especially in unselected TBI populations.[30]

It has been suggested that in moderate and severe TBI, outcome is determined by what ''the injury brings to the patient'' whereas in mTBI it is what ''the patient brings to the injury,'' and our data support this statement. Generally accepted prognostic models for moderate and severe TBI include, in addition to age, indicators of injury severity, such as GCS, pupillary reactivity, and CT parameters.[9,10,26] These predictors are less relevant in mTBI. Here, indicators of social background, history of psychiatric conditions, assault as cause of injury, and low education seem to be predictive of poorer outcome. However, the combination of pre-existing psychiatric conditions, low education, and assault as a cause of injury as predictors of 6-month outcome poses the question of whether persistent complaints are fully attributable to the TBI. Future studies that follow up with more-sensitive and -specific outcome measures in larger cohorts are required to answer this question. In this study, we neither aimed nor had enough patients to fully disentangle the mechanisms causing poor outcome. This would be essential to target treatment to patients at high risk for poor outcome and should be a main focus of future studies and large ongoing efforts such as CENTER-TBI and TRACK-TBI.

The predictors we combined in our multi-variable analysis had a moderate discriminative ability (AUROCs, 0.68''0.69). Emerging technologies that could improve prognostication in mTBI include proteomic biomarkers,[31–33] genetic factors,[34–36] and improved imaging biomarkers, including magnetic resonance imaging.[37] Additionally, prediction models for mTBI may require more-sensitive and -specific outcome measures beyond the GOS-E.

We recognize several limitations to our study. We included patients with GCS 13–15, which are classified in the category of mTBI. However, there were patients with one or two unreactive pupils, an AISh of 4 or 5, or a Marshall's CT classification of 5 or 6 (indicative of ''complicated'' mTBI with pathological head CT findings), all indicating quite severe injury. More than half of the patients reported previous head injury, which may be an overestimation given that it was self-reported without necessarily requiring hospital admission. Pre-existing psychiatric conditions proved to be one of the strongest predictors to poorer outcome. A goal of the TRACK-TBI Pilot Study was to evaluate the feasibility of implementing the TBI CDEs V1.0, which did not include a validated structured interview for preinjury psychiatric history. Even though we implemented the highest level of granularity for baseline data collection, we were unable to capture the specific types, durations, and formal diagnoses of pre-existing psychiatric conditions. In moving forward, establishing a standard set of tools and questionnaires to obtain this level of granularity will be helpful in evaluating the true associations among pre-existing mental health conditions and post-TBI outcome.

Conclusion

Reliable outcome prediction in mTBI is important for clinical practice. Identifying patients at increased risk of unfavorable outcome permits targeting closer observation and early intervention, which may reduce the psychological burden of injury on patients, as well as the related economic burden on society. Our study demonstrates that existing models for mTBI perform unsatisfactorily. We tested 21 variables in ordinal analysis of 386 patients, which is 1 in 18 and thus reasonable from a statistical perspective. Although we have found some strong predictors of poor outcome, such as age and history of psychiatric condition, given the sample size, we consider the results of our prognostic analysis as hypothesis generating. These predictors will need further validation in ongoing prospective, longitudinal studies, such as those that are part of the International TBI Research Initiative.[38,39]

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Author Disclosure Statement

No competing financial interests exist.

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Original Articles

Measurement of the Glial Fibrillary Acidic Protein and Its Breakdown Products GFAP-BDP Biomarker for the Detection of Traumatic Brain Injury Compared to Computed Tomography and Magnetic Resonance Imaging

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Abstract

Glial fibrillary acidic protein and its breakdown products (GFAP-BDP) are brain-specific proteins released into serum as part of the pathophysiological response after traumatic brain injury (TBI). We performed a multi-center trial to validate and characterize the use of GFAP-BDP levels in the diagnosis of intracranial injury in a broad population of patients with a positive clinical screen for head injury. This multi-center, prospective, cohort study included patients 16–93 years of age presenting to three level 1 trauma centers with suspected TBI (loss of consciousness, post-trauma amnesia, and so on). Serum GFAP-BDP levels were drawn within 24 h and analyzed, in a blinded fashion, using sandwich enzyme-linked immunosorbent assay. The ability of GFAP-BDP to predict intracranial injury on admission computed tomography (CT) as well as delayed magnetic resonance imaging was analyzed by multiple regression and assessed by the area under the receiver operating characteristic curve (AUC). Utility of GFAP-BDP to predict injury and reduce unnecessary CT scans was assessed utilizing decision curve analysis. A total of 215 patients were included, of which 83% suffered mild TBI, 4% moderate, and 12% severe; mean age was 42.1 ± 18 years. Evidence of intracranial injury was present in 51% of the sample (median Rotterdam Score, 2; interquartile range, 2). GFAP-BDP demonstrated very good predictive ability (AUC = 0.87) and demonstrated significant discrimination of injury severity (odds ratio, 1.45; 95% confidence interval, 1.29–1.64). Use of GFAP-BDP yielded a net benefit above clinical screening alone and a net reduction in unnecessary scans by 12–30%. Used in conjunction with other clinical information, rapid measurement of GFAP-BDP is useful in establishing or excluding the diagnosis of radiographically apparent intracranial injury throughout the spectrum of TBI. As an adjunct to current screening practices, GFAP-BDP may help avoid unnecessary CT scans without sacrificing sensitivity (Registry: ClinicalTrials.gov Identifier: NCT01565551).

Key words: biomarkers; imaging; traumatic brain injury

Introduction

Clinical care and research in traumatic brain injury (TBI) rely on classification systems, such as the Glasgow Coma Scale (GCS), that are not adequately calibrated for injury assessment across mild and moderate TBI.[1] Radiographic evaluation is central to the initial stratification of injury severity and to monitor for acute changes; however, its use is limited by cost and perceived risk of ionizing radiation.

Simpler, sensitive, and specific tests for identifying and stratifying TBI would provide more rapid and tailored diagnosis of TBI

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while minimizing the time, risk, and cost associated with current standards. To this end, there has been increasing investigation into serum proteins as biomarkers of TBI; however, none have yet been validated for routine use. Potential biomarkers under investigation include glial protein S-100 beta (S100B), neuron-specific enolase (NSE), myelin basic protein, ubiquitin c-terminal hydrolase, and glial fibrillary acid protein (GFAP).[2,3] GFAP, initially investigated in the 1970s, has emerged as a promising biomarker candidate to improve diagnosis, triage, and targeted treatment of TBI patients.[4] GFAP is an intermediate filament protein component of the astrocyte cytoskeleton expressed almost exclusively in the central nervous system (CNS). While insoluble in intact astrocytes, overactivation of calpain after initial injury and gliolysis produce soluble GFAP polymers (or breakdown products) that are released into interstitial fluid.[5] These GFAP breakdown products (GFAP-BDP) can be measured in serum in association with a number of CNS disorders, including TBI.[1,2] Previous studies have correlated elevated GFAP-BDP with the presence of clinical and radiographic injury as well as worse outcome and need for neurosurgical intervention.[2,3] To date, previous work has focused primarily on the severe TBI population or compared TBI patients against either uninjured patients or those not meeting clinical criteria for head injury. Our previous study was one of the first to prospectively assess GFAP-BDP with regard to presence and severity of radiographic injury on computed tomography (CT) across the entire spectrum of disease after TBI.[4,6]

The aim of this study was to evaluate and validate the utility of GFAP-BDP for the diagnosis of intracranial injury in patients with a positive clinical screen for head injury across the spectrum of TBI typically presenting to a level 1 trauma center. We expand on our previous analysis of the utility of GFAP-BDP to identify TBI, including injury evaluation by MRI, cut-off values for GFAP-BDP specifically in the mild and moderate TBI groups, and analysis of the potential reduction of CT scans by utilizing the biomarker for injury detection.[6]

Methods

Study population

Recruitment of subjects was part of the TRACK-TBI (Transforming Research and Clinical Knowledge in Traumatic Brain Injury) Pilot Study, a National Institute of Neurological Disorders and Stroke–funded, multi-center, prospective collaboration among three U.S. level 1 trauma centers enrolling acute TBI patients (University of Pittsburgh Medical Center [UPMC]; University Medical Center Brackenridge [UMCB]; and University of California, San Francisco [UCSF]) and one rehabilitation center (Mount Sinai Rehabilitation Center) enrolling late-presenting TBI patients to develop, test, and refine TBI common data elements (TBI-CDEs) for research across four major domains: demographics, neuroimaging, biomarkers, and outcome measures.[7] The TBI population under investigation spanned the entire injury spectrum, from severe to mild. Both patients with negative imaging and those discharged from the emergency department (ED) are also included in the total population. Institutional review boards of participating centers approved all study protocols. All participants or their legal authorized representatives gave written informed consent. At follow-up, participants previously consented by legal authorized representative, if neurologically improved to be cognizant, were consented for continuation in the study.

To be eligible for this analysis, patients must have presented to an ED within 24 h of their injury and had a positive clinical screen for acute TBI necessitating a noncontrast head CT according to American College of Emergency Physicians/Centers for Disease Control and Prevention (ACEP/CDC) evidence-based joint practice guidelines.[8] These guidelines represent an amalgam of the Canadian CT Head Rule and the New Orleans Criteria (Haydel, Indications for computed tomography in patients with minor head injury; Stiell, The Canadian CT Head Rule for patients with minor head injury). GCS score was assessed by a neurosurgeon at admission and was reconfirmed by study personnel at the time of biomarker collection. TBI severity was broadly defined by GCS, with mild between 13 and 15, moderate between 9 and 12, and severe between 3 and 8. Patients were excluded if they were younger than 16 or greater than 95 years of age, suffered penetrating head injury, or had a premorbid neurologic condition.

Sample collection and measurement of glial fibrillary acidic protein and its breakdown products

Data from the three level 1 trauma centers were used for this analysis. Serum samples were collected within 24 h of injury and were dated and time stamped to compare with time of injury. The TBI-CDE Biospecimens and Biomarkers Working Group Guidelines for sample preparation were followed.[9] Samples were centrifuged and serum aliquots stored at $-80°C$ for future batch processing. UPMC and UMCB batch-shipped samples, overnight on dry ice, to UCSF. All deidentified samples were then stored with a unique study number specific to site and subject. A central database was maintained by the coordinating center (UCSF) with each site entering site-specific data for final statistical reporting. Blinded sample analysis occurred in a single laboratory (Banyan Biomarkers, Alachua, FL) using a sandwich enzyme-linked immunosorbent assay (ELISA) to GFAP-BDP. The GFAP ELISA utilized a proprietary mouse monoclonal antibody for solid-phase immobilization, and a proprietary polyclonal rabbit antibody for detection.[10,11] Testing procedure and detection of GFAP was carried out as previously described.[6] Both whole GFAP molecules as well as GFAP-BDPs are detected by the assay, potentially resulting in a more complete measure of overall GFAP released into circulation. All samples were analyzed in duplicate concomitantly with calibrators prepared in compatible matrix, as described previously.[6] From high concentration to low, the previously reported intraassay coefficient of variance for the ELISA is 4.3–7.8% and the interassay coefficient of variance is 7.8–14.3%. The estimated limit of detection for GFAP is ~ 0.01 ng/mL.[11]

Evaluation of endpoints

All patients underwent CT imaging of the brain at the time of initial presentation to the ED. Patients were offered a follow-up, out-patient MRI upon enrollment in the TRACK-TBI study. The MRI was on a voluntary, opt-in basis to be performed 1–2 weeks postinjury. Radiographic images were deidentified, uploaded to a central imaging database, and reviewed by a blinded central reader. Imaging features were extracted and entered into the TRACK-TBI database. Each patient's head CT and magnetic resonance image (MRI) were characterized using the recommendations of the TBI-CDE Neuroimaging Working Group regarding specific radiologic features, data definitions needed to characterize injuries, and best practices needed to optimize and harmonize imaging data acquisition for TBI research during data collection.[12,13] Specifically, the presence of cisternal effacement, mid-line shift, epidural hematoma, subarachnoid hemorrhage, and intraventricular hemorrhage were recorded to determine the Rotterdam score for all scans (assessment of TBI severity based on noncontrast head CT). The presence of any intracranial abnormalities on MRI was considered a positive scan. Imaging studies were performed at the discretion of each study site using their standard equipment and protocols.

The primary endpoint for analysis was intracranial injury, as identified on CT scan at time of presentation. Secondary endpoints included severity of intracranial injury, as measured by the

Rotterdam score, and presence of intracranial injury, as identified by delayed MRI.

Statistical analysis

Continuous demographic characteristics were assessed for normality using the Kolmogorov-Smirnov's test; normally distributed data were analyzed by *t*-test, whereas the remainders were compared using the Wilcoxon's rank-sum test. Categorical data were analyzed by Pearson's chi-squared or Fisher's exact test. Differences between groups in multi-level ordinal measurements (i.e., Rotterdam score, GCS, and Glasgow Outcome Scale) were tested using Kruskal-Wallis' test. Univariable regression analysis was performed to assess the association between GFAP-BDP level and radiographic presence of intracranial injury. Multi-variate regression models were later built to evaluate the predictive capabilities GFAP-BDP after adjustment for known factors associated with severity of intracranial injury (age, pupillary reactivity, GCS, and Injury Severity Score [ISS]). The ability of GFAP-BDP to predict severity of intracranial injury was assessed using ordered logistic regression modeling.

The ability of GFAP-BDP to predict the presence of intracranial injury was analyzed apropos of accuracy, discrimination, calibration, and clinical utility. Discrimination was assessed using the area under the receiver operating characteristic (ROC) curve (AUC). Using current statistical consensus, AUCs of 0.8–0.9 are considered very good, 0.7–0.8 as adequate, and below 0.7 as poor. Calibration was tested with the Hosmer-Lemeshow's goodness-of-fit test. Cut-off values for GFAP-BDP were assessed both for the highest accuracy and for the highest specificity, specifically in the mild to moderate injury groups. Values were determined utilizing ROC curves and AUC and Brier scores were calculated. Clinical utility was evaluated by decision curve analysis.[14] Statistical significance was set at $p < 0.05$. All data were analyzed using STATA statistical software (12; StataCorp LP, College Station, TX).

Results

Baseline demographics

A total of 215 patients were available for analysis. Demographic characteristics are shown in Table 1. Mean age was 42 ± 18 years, with a minimum of 16 and maximum of 93 years. Approximately 73% of patients were male. Median GCS for the entire sample was 15 (interquartile range [IQR], 1), with mild TBI (GCS, 13–15) constituting 83% (GCS,13–15), moderate 4% (GCS, 9–12), and severe 13% (GCS, 3–8). Seventy percent of patients had a documented loss of consciousness (LOC), whereas 38% had documented post-traumatic amnesia (PTA). Median Injury Severity Score (ISS) was 10 (IQR, 17), with 36% suffering significant polytrauma (ISS, ≥ 16). Mean GFAP-BDP was 1.59 ± 2.98 ng/mL, and minimum and maximum levels detected were 0.02 and 20.1 ng/mL, respectively. Pair-wise correlation between CT and MRI was 0.33 ($p = 0.0096$). There was no significant correlation between MRI and Rotterdam score.

Glial fibrillary acidic protein and its breakdown products and computed tomography outcomes

Fifty-one percent ($n = 110$) of patients presenting with positive clinical screen for TBI had intracranial pathology demonstrated on admission CT. Median Rotterdam score of this cohort was 3 (IQR, 1). Serum level of GFAP-BDP was significantly higher in those with CT-positive intracranial injury, compared to those without (2.86 ± 3.74 vs. 0.26 ± 0.41 ng/mL, respectively; $p < 0.001$). Figure 1 presents a box plot of GFAP-BDP values for the two patient cohorts. Univariable analysis demonstrated elevated GFAP-BDP level and conferred significant risk of intracranial injury on initial CT (odds ratio [OR], 8.9; 95% confidence interval [CI], 2.3–2.5; $p < 0.001$), as also demonstrated in our previous study.[6] Further, elevated GFAP-BDP remained a significant predictor after adjustment for known predictors of intracranial injury severity and functional outcome (i.e., age, pupillary activity, GCS, and ISS; OR, 5.5; 95% CI, 2.00–14.9; $p < 0.001$).

Figure 2 shows GFAP-BDP levels in relation to radiographic injury severity classification according to Rotterdam score. Level of GFAP-BDP differed significantly as a function of Rotterdam score ($p < 0.001$). Ordinal regression analysis revealed that elevated GFAP-BDP level significantly predicted worse Rotterdam score, both independently (OR, 1.20; 95% CI 1.1–1.3) as well as after adjustment for age, GCS, and ISS (OR, 1.17 95% CI, 1.1–1.3; $p < 0.001$).

GFAP-BDP level was the most accurate predictor of the presence or absence of intracranial injury detected by radiographic imaging (accuracy, 81%), as compared with accepted clinical predictors of intracranial injury (age, 65%; GCS, 62%; LOC and/or

Table 1. Baseline Demographic Characteristics at Time of Admission by Presence of Intracranial Injury on CT

Baseline characteristics	*All* (n = *215*)	*CT negative* (n = *105*)	*CT positive* (n = *110*)	p *value*
Age, mean ± SD (years)	42 ± 18	37 ± 16	47 ± 18	< 0.01
Sex, % male	73 (156)	69 (72)	76 (84)	0.22
GCS, median (IQR)	15 (1)	15 (0)	15 (4)	< 0.01
Mild, % 13–15	83 (179)	97 (102)	70 (77)	
Moderate, % 9–12	4 (9)	2 (2)	6 (7)	
Severe, % 3–8	13 (27)	1 (1)	24 (26)	
Pupillary reactivity, %				< 0.01
Both	94 (202)	100 (105)	88 (97)	
Anisocoria	2 (4)	—	4 (4)	
Unreactive	4 (9)	—	8 (9)	
ISS, median (IQR)	10 (17)	0 (4)	17 (12)	< 0.01
Polytrauma, % ISS ≥ 16 (*n*)	36 (78)	5 (5)	66 (73)	< 0.01
Rotterdam score, median (IQR)		—	3 (1)	
GFAP-BDP, mean ± SD (ng/mL)	1.59 ± 2.98	0.26 ± 0.41	2.86 ± 3.74	< 0.01

CT, computed tomography; GCS, Glasgow Coma Score; ISS, Injury Severity Score; SD, standard deviation; IQR, interquartile range; GFAP-BDP, glial fibrillary acidic protein and its breakdown products.

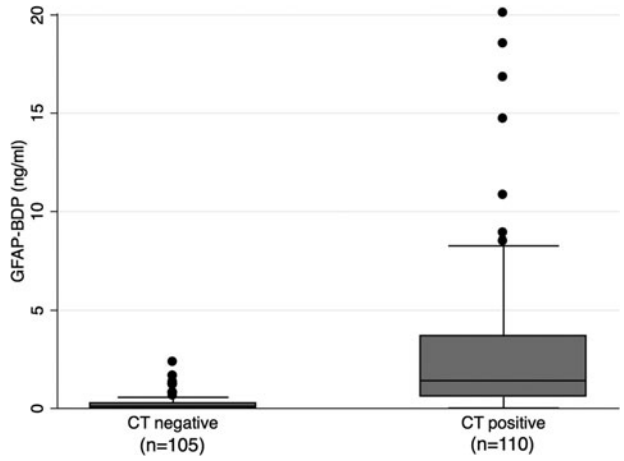

FIG. 1. Box plots showing median levels of GFAP-BDP measured on admission in two groups of patients. Boxes show interquartile ranges, and I bars represent highest and lowest values. CT, computed tomography;GFAP-BDP, glial fibrillary acidic protein and its breakdown products.

PTA, 54%; pupillary status, 52%). In our sample, accuracy of GFAP-BDP for injury prediction was superior to the ACEP/CDC recommended criteria for neuroimaging in TBI (81% vs. 65%, respectively).[8] Discriminatory analysis of GFAP-BDP resulted in an AUC of 0.88 (95% CI, 0.83–0.93), indicating very good discriminatory ability. Level of GFAP-BDP retained its discriminatory value after adjustment for age, pupillary exam, GCS, and ISS (AUC, 0.96; 95% CI, 0.7–0.91; Fig. 3). Calibration analysis did not show systematic error across risk deciles ($p = 0.15$). Calculation of a cut-off value to maximize accuracy in the mild and moderate injury range specifically yielded a GFAP-BDP level of 0.6 ng/mL, with a sensitivity of 67%, a specificity of 89%, and a Brier score of 0.21. A cut-off value to maximize specificity was calculated at a GFAP-BDP concentration of 1.66 ng/mL, resulting in a sensitivity of 45%, specificity of 99%, and a Brier score of 0.29.

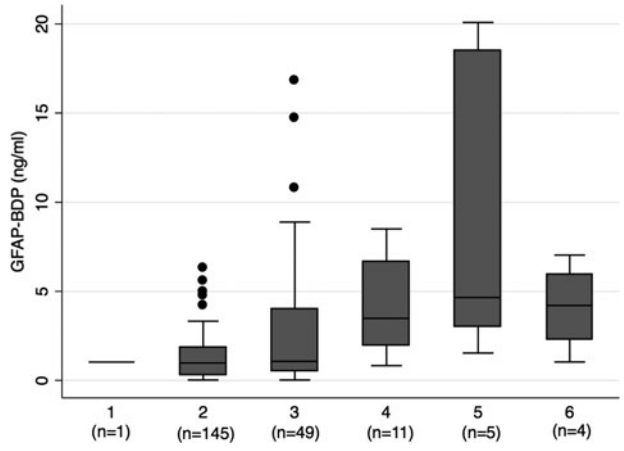

FIG. 2. Box plots showing median levels of GFAP-BDP measured on admission among patients in each of the Rotterdam classifications of injury on CT. Boxes show interquartile ranges, and I bars represent highest and lowest values. Overall, GFAP-BDP was significantly different across each level of Rotterdam score ($p \leq 0.001$). CT, computed tomography; GFAP-BDP, glial fibrillary acidic protein and its breakdown products.

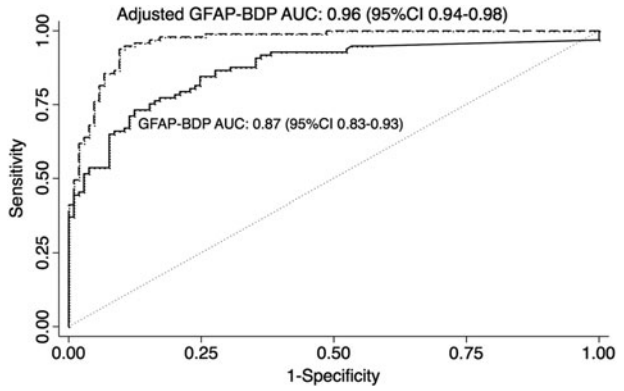

FIG. 3. Receiver-operating-characteristic curves for various cut-off levels of GFAP-BDP in differentiating presence or absence of intracranial injury on CT. Curves for GFAP-BDP alone and after adjustment for known predictors of injury and severity (age, GCS, pupillary reactivity, and ISS). AUC, area under the receiver operating characteristic curve; CI, confidence intreval; CT, computed tomography; GCS, Glasgow Coma Scale; GFAP-BDP, glial fibrillary acidic protein and its breakdown products; ISS, Injury Severity Scale.

Clinical utility of GFAP-BDP was evaluated through decision curve analysis as an extension of currently established practice guidelines.[15] Decision curves are displayed in Figure 4. Use of GFAP-BDP displayed superior net benefit, as compared to scanning all patients with a positive clinical screen for head injury beginning at a threshold probability (i.e., perceived risk of injury) of approximately 20% or higher. This correlated to a net reduction of 12 CT scans per 100 patients without missing a single injury (12% reduction in unnecessary imaging). Reduction of unnecessary scans increased to 18% when applied to patients with a perceived risk of injury of 25% and by more than 30% if the risk of injury was equivalent to the prevalence of injury in this sample (CT-positive after clinical screen, $\sim 51\%$).

Glial fibrillary acidic protein and its breakdown products and magnetic resonance imaging outcomes

Sixty patients underwent MRI in the subacute injury phase; of these, 35% ($n = 21$) had positive scans (see Table 2). Of note, MRI revealed injuries in 13 patients who had had negative CT imaging on initial evaluation. Further, 4 patients with positive CT scans had negative follow-up findings on MRI. There was no significant difference between MRI-positive and -negative patients in age, gender, pupillary status, GCS, ISS, or functional outcome (Glasgow Outcome Scale Extended at 6 and 12 months). Admission GFAP-BDP values were significantly higher in MRI-positive patients (1.31 ± 1.8 vs. 0.28 ± 0.57 ng/mL, respectively; $p = 0.001$). In univariable analysis, GFAP-BDPs significantly predicted the presence of intracranial pathology, as observed on MRI (OR, 2.7; 95% CI, 1.2–5.7). GFAP-BDP remained an independent predictor of injury on MRI after multivariate analysis, adjusting for age, pupillary status, GCS, and ISS (OR, 3.8; 95% CI, 1.3–11.3). Post-hoc, subgroup analysis performed on CT-negative, MRI-positive patients, in comparison with the remainder of the CT-negative cohort (35 patients), did not demonstrate significant differences in age, GCS, ISS, or GFAP-BDP levels.

Analysis of GFAP-BDP for the prediction of injury on MRI demonstrated an accuracy of 72%, adequate discrimination of 0.70

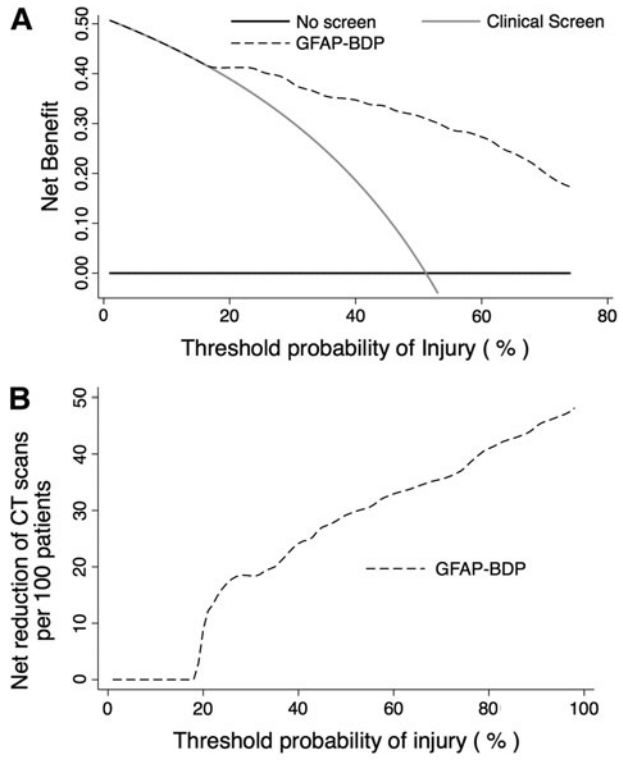

FIG. 4. (**A**) Decision curve analysis of the net benefit of GFAP-BDP to predict injury compared to current clinical screening method or scanning all patients regardless of screening across various probabilities of injury. (**B**) Decision curve analysis of the reduction of unnecessary CT scans per 100 patients using GFAP-BDP as an adjunct to predict injury compared to current clinical screening methods across various probabilities of injury. CT, computed tomography; GFAP-BDP, glial fibrillary acidic protein and its breakdown products.

(AUC; 95% CI, 0.55–0.85), and adequate calibration ($p = 0.41$). Decision curve analysis demonstrated that GFAP-BDP contributes a net benefit above an injury-risk threshold of 25%, with a 13% reduction in unnecessary scans. Utilization of the cut-off value of 0.6 ng/mL in the mild-to-moderate range of injury was calculated to have a net benefit at an injury threshold of 24% and an overall net reduction in CT scans of 30 per 100 patients in this group.

Table 2. Baseline Demographic Characteristics at Time of Admission by Presence of Intracranial Injury on MRI

Baseline characteristics	*MRI negative (n = 39)*	*MRI positive (n = 21)*	p *value*
Age, mean ± SD (years)	39 ± 17	42 ± 15	0.32
Sex, % male	64 (25)	76 (16)	0.33
GCS, median (IQR)	15 (0)	15 (0)	0.68
ISS, median (IQR)	0 (0)	0 (10)	0.12
GFAP-BDP, mean ± SD (ng/mL)	0.28 ± 0.57	1.31 ± 1.77	< 0.01

MRI, magnetic resonance imaging; GCS, Glasgow Coma Score; ISS, Injury Severity Score; SD, standard deviation; IQR, interquartile range; GFAP-BDP, glial fibrillary acidic protein and its breakdown products.

Discussion

This multi-center, prospective study demonstrates that serum measurement of GFAP-BDP as a biomarker possesses the necessary characteristics (accuracy, discrimination, calibration, and clinical utility) for improved prediction of radiographically evident injury across the spectrum of TBI. Additionally, GFAP-BDP levels were able to discriminate severity of intracranial injury independent of other classic injury predictors. GFAP-BDP also accurately predicted persistence of intracranial injury on imaging performed in the subacute period, again independent of other markers of injury risk. These data expand upon our previous study demonstrating a correlation between injuries observed on CT scan and elevated levels of GFAP-BDP.[6] Taken together, these results indicate that GFAP-BDP is a viable early indicator of intracranial injury and represents a useful adjunct to current diagnostic methods for TBI.

Numerous serum biomarker candidates for the diagnosis of TBI have come under intense scrutiny; however, none to this point have demonstrated sufficient utility to justify routine clinical use. Studies have reported a consistent correlation between elevated serum levels of S-100B and GCS, radiographic findings, and outcome.[16] Despite its sensitivity, S-100B has been shown to be elevated in trauma patients without head injury, as well as after hemorrhagic shock and in normal pediatric patients.[16] This lack of specificity limits its possible diagnostic practicality. Similarly, NSE, although rapidly elevated post-TBI, is also found in states of hemolysis.[17] GFAP-BDP is a product of astrocyte cytoskeleton degradation by calpain protease activation and therefore considered specific to the CNS. This has already been corroborated by a number of studies evaluating levels after TBI, compared to noninjured controls, as well as those suffering only traumatic extracranial injuries.[11,18] This study further supports the specificity of GFAP-BDP to detect radiographically evident injury given that predictive ability was evaluated among patients with similar clinical scenarios and presenting neurological exams. Against this clinically relevant sample, GFAP-BDP remained a sensitive and specific predictor of injury even after adjustment for the presence of polytrauma (i.e., ISS).

Previous evaluations of GFAP-BDP, largely focusing on severe TBI, have demonstrated a correlation between elevated marker levels and injury severity, number of lesions, and mortality.[19] More recently, Papa and colleagues specifically studied GFAP-BDP within the mild-to-moderate TBI population and found that GFAP-BDP adequately predicted presence of injury, severity of injury, and need for neurosurgical intervention.[11] The current study evaluates GFAP-BDP across the entire spectrum of TBI, in the context of all patients who screen positive for intracranial injury using established guidelines. Alone, GFAP-BDP demonstrated the highest accuracy among predictors and very good discrimination (AUC, 0.88). Importantly, despite varied injury states and severity, calibration did not demonstrate systematic errors, further supporting the use of GFAP-BDP across severity cohorts. Importantly, GFAP-BDP also independently predicted the degree of radiographic injury throughout the spectrum of presenting neurological exams. This correlation supports the idea that GFAP release, breakdown, and translocation to serum mirrors radiographic evidence of parenchymal injury and disruption of the blood–brain barrier.

Pressure to deliver cost-effective care and concern over the potential effects of unnecessary ionizing radiation have prompted more judicious use of CT imaging for the evaluation of head injury. Despite the implementation of the Canadian CT Head Rule and/or New Orleans Criteria to stratify patients, approximately 60–90% of

patients imaged for head injury will have a negative CT.[20] Biomarkers, ideally, could act as adjuncts to these validated approaches, to better and more cost-efficiently classify at-risk patients. To assess clinical utility in this context, we analyzed GFAP-BDP utilizing decision curve analyses to determine the probability of injury above which GFAP-BDP benefits diagnosis without increasing unnecessary scans. This study found that use of GFAP-BDP has a superior net benefit from a threshold probability of injury of 20% and greater. This suggests that measuring serum GFAP-BDP, in conjunction with current practice guidelines, would lead to a 12% reduction in unnecessary imaging at this relatively low-risk threshold for injury (common probability thresholds for cancer and cardiac screening are 10–20%). Specifically in the mild to moderate groups, where there is the most potential benefit from a reduction in CT scans, we calculated that, at a concentration of 0.6 ng/mL, there is a net benefit at an injury probability threshold of 24% with a potential reduction in scans of 30 per 100 patients. When used as an adjunct to ACEP Guidelines, GFAP-BDP would reduce unnecessary CT scans by greater than 20% at a risk threshold of 25%, and by more than 30% in a population with a prevalence of injury similar to our sample ($\sim$51%).[8] Currently only 6–10% of patients with GCS 13–15 have lesions detected on CT scan, and only 0.4–1% of these require neurosurgical intervention, indicating that many patients may not need imaging if other reliable and accurate options for injury detection are available.[21] With approximately 1.5 million patients diagnosed as sustaining a mild TBI, estimating 80% receive a CT scan, and an average cost of $216 per CT scan, a reduction in scans of 30% could yield a potential savings of $77.8 million dollars per year in this population.[22,23]

There are several limitations to our study. GFAP-BDP was only measured at initial presentation and thus levels were unable to be trended to evaluate whether decreasing GFAP-BDP correlates with injury resolution or to track the trend in concentration over time. This precluded analysis of changes in concentration of GFAP-BDP over time as compared to evolution of injury on imaging. Our analysis included only those patients who received a head CT as part of enrollment in the TRACK-TBI study, and we therefore had a relatively high number of mild TBI patients with positive findings on CT scan. This may have excluded the less severely injured patients from GFAP-BDP measurement. Additionally, our analysis was limited to the clinical indicators of injury as defined by the TRACK-TBI study, and we were unable to compare GFAP-BDP against the numerous indicators of intracranial injury that may otherwise be used. We also were unable to include cost data on serum analysis for GFAP-BDP concentrations given that the data are publicly not available and remain confidential owing to the fact that the test is not yet U.S. Food and Drug Administration approved for clinical use. Therefore, we were unable to provide further analysis as to potential cost savings compared to CT scans. This is the first study, to our knowledge, to evaluate the performance of GFAP-BDP against the Rotterdam score and against positive findings on MRI. However, MRI data were collected on an opt-in basis at up to 2 weeks postinjury, potentially biasing this cohort to include patients with more-severe or persistent symptoms. This may help to account for the lower discriminatory ability of GFAP-BDP among MRI patients; nonetheless, GFAP-BDP remained a significant predictor after adjustment.

This analysis demonstrates that GFAP-BDP can reliably detect the presence of injury on radiographic imaging as well as predict injury severity across the spectrum of TBI. Early measurement of GFAP-BDP can contribute to more-accurate diagnosis and triage of TBI patients, decreasing the number of unnecessary CT scans and allowing more tailored management of the brain injury.

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Author Disclosure Statement

No competing financial interests exist. No conflicts of interest.

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RESEARCH ARTICLE

Uncovering precision phenotype-biomarker associations in traumatic brain injury using topological data analysis

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Abstract

Background

Traumatic brain injury (TBI) is a complex disorder that is traditionally stratified based on clinical signs and symptoms. Recent imaging and molecular biomarker innovations provide unprecedented opportunities for improved TBI precision medicine, incorporating patho-anatomical and molecular mechanisms. Complete integration of these diverse data for TBI diagnosis and patient stratification remains an unmet challenge.

Methods and findings

The Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Pilot multicenter study enrolled 586 acute TBI patients and collected diverse common data elements (TBI-CDEs) across the study population, including imaging, genetics, and clinical outcomes. We then applied topology-based data-driven discovery to identify natural subgroups of patients, based on the TBI-CDEs collected. Our hypothesis was two-fold: 1) A machine learning tool known as topological data analysis (TDA) would reveal data-driven patterns in patient outcomes to identify candidate biomarkers of recovery, and 2) TDA-identified biomarkers would significantly predict patient outcome recovery after TBI using more traditional methods of univariate statistical tests. TDA algorithms organized and mapped the data of TBI patients in multidimensional space, identifying a subset of mild TBI patients with a specific multivariate phenotype associated with unfavorable outcome at 3 and 6 months

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after injury. Further analyses revealed that this patient subset had high rates of post-traumatic stress disorder (PTSD), and enrichment in several distinct genetic polymorphisms associated with cellular responses to stress and DNA damage (PARP1), and in striatal dopamine processing (ANKK1, COMT, DRD2).

Conclusions

TDA identified a unique diagnostic subgroup of patients with unfavorable outcome after mild TBI that were significantly predicted by the presence of specific genetic polymorphisms. Machine learning methods such as TDA may provide a robust method for patient stratification and treatment planning targeting identified biomarkers in future clinical trials in TBI patients.

Trial Registration

ClinicalTrials.gov Identifier NCT01565551

Introduction

Traumatic brain injury (TBI) annually produces 52,000 deaths, 257,000 hospitalizations and 2.2 million emergency visits in the United States (US) alone [1]. Even though TBI is a major cause of death and disability, it is currently diagnosed with crude, symptom-based tools, and few targeted treatments exist. During the initial TBI event, biomechanical forces interact with complex tissue geometry to produce nonlinear microforces, resulting in distributed multifocal lesions throughout the brain [2]. At a cellular level TBI results in membrane disruption, cell death, and diffuse axonal injury, accompanied by a cascade of secondary injury mechanisms that evolve over time [3,4]. These complex biological processes produce a poorly understood constellation of clinical symptomatology, with multifaceted impairments ranging from motor deficits to debilitating neurocognitive and personality changes. Because of the significant, multimodal heterogeneity of post-TBI symptoms, post-event treatment and follow-up pose a significant challenge. Some of these impairments may even go undiagnosed, particularly in the milder categories of TBI that include concussion.

One approach to better understand and to treat symptoms of TBI is to identify biomarkers for vulnerable patient subpopulations. However, defining clear central nervous system (CNS) biomarkers has historically been challenging, given the heterogeneity of TBI [5]. Fortunately, recent innovations in molecular biology and imaging provide unprecedented opportunities for data-rich phenotyping [6]. To aid TBI precision medicine, the National Institutes of Health-National Institute of Neurological Disorders and Stroke (NIH-NINDS) launched a major initiative to define TBI common data elements (TBI-CDEs). Hence, there is now a concerted and collaborative effort among researchers to define, collect, and analyze TBI-CDEs. The multicenter Transforming Research and Clinical Knowledge in TBI Pilot (TRACK-TBI Pilot) study provides the first prospective test of the feasibility and utility of the NINDS TBI CDEs [6,7]. As part of the effort, TRACK-TBI Pilot developed an information commons from the CDEs collected prospectively for 586 acute TBI patients from 3 level 1 trauma centers in the US.

Given the highly detailed and multi-scalar data in TRACK-TBI Pilot, we approached the problem from a model-free perspective, using an approach that has been developed from the

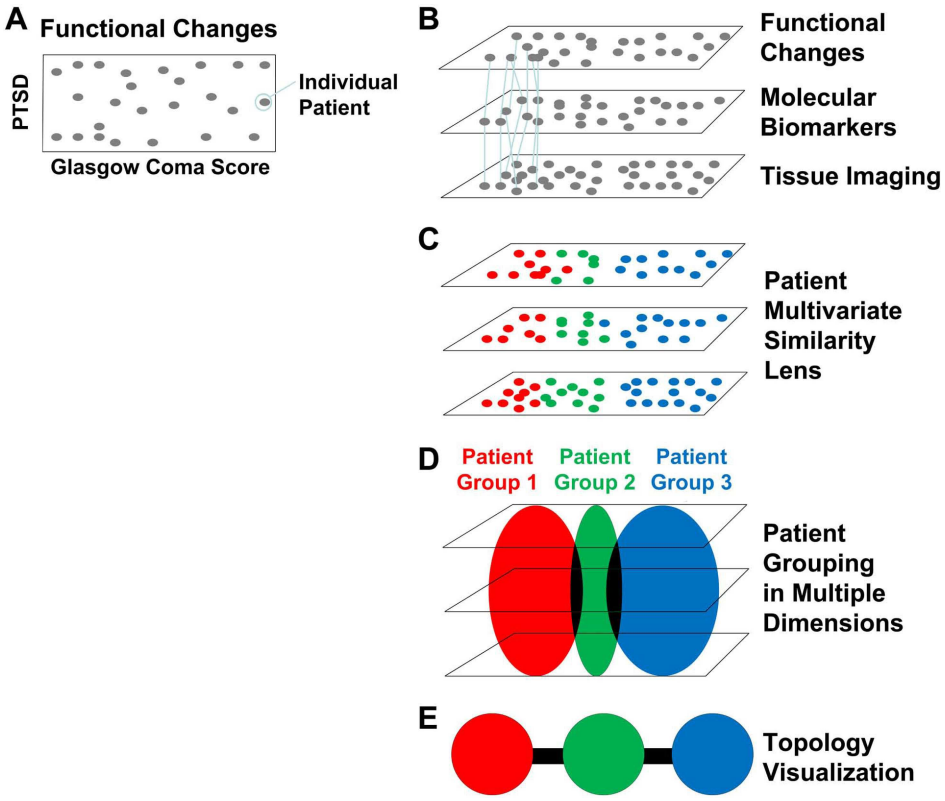

Fig 1. (A-E). Methodological work-flow for integrating diverse clinical TBI data. (A) Hypothetical example of a spatial bi-plot of individual patients (grey points) on 2 functional endpoints after TBI (GOS-E AND PTSD). The same approach can be applied to multiple metrics simultaneously using multivariate pattern detectors (e.g., principal component analysis) to produce a multivariate view of function. (B) In TRACK-TBI Pilot the same individuals (N = 586) were tracked prospectively across multiple domains (function, biomarkers, imaging) providing connections (lines) across domains to improve patient classification using the full syndromic space. (C) Multivariate pattern detection lens can be used to categorize (colors) patients across all domains. (D) Patient grouping by multivariate lens. (E) Topological visualization renders patient groups into individual nodes, colored by the multivariate lens. Edges (black lines) indicate individuals appearing in both groups producing a syndromic map of patient clusters.

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application of TDA on real-world datasets [8–10]. TDA is a machine learning data analytic used to cluster patients based on functional outcome data to derive novel insights into disease mechanisms (Fig 1). To date TDA has been successfully applied to biological datasets to discover novel insights including identification of subpopulations of cancer, identification of genomic biomarkers, disease association, RNA folding, viral evolution, immunology, diabetes, and preclinical spinal cord injury and TBI[10–16]. The current study aims to test the following hypotheses in the TRACK-TBI Pilot information commons: 1) TDA will reveal data-driven patterns in patient outcomes to identify candidate biomarkers of recovery following TBI, and 2) TDA-identified biomarkers predict patient outcome recovery after TBI.

Methods

TBI Common Data Elements (TBI-CDEs) and the TRACK-TBI pilot study

The NIH/NINDS developed the TBI-CDEs to overcome pitfalls in TBI clinical research, including lack of standardization in data collection and analysis, inability to appropriately stratify patients, and discordant injury types [17]. Using a consensus-based approach, NINDS

working groups developed standards for data capture across 4 broad domains: clinical assessments and demographic information, genetics and proteomics, neuroimaging, and outcome measures. The NINDS-CDE planning committee instructed working groups to stratify data elements into 1 of 3 categories: 'core', 'basic,' and 'supplemental.'[18] Core elements comprise the most basic information: data that is absolutely fundamental to capture (e.g., gender, age). Basic elements provide additional diagnostic detail (e.g., education level, cause of injury). Emerging CDEs include innovative approaches that require validation before broad clinical adoption (e.g. imaging, serial plasma biomarkers).) [19]. The multicenter prospective TRACK-TBI Pilot study assessed the feasibility and utility of the TBI-CDEs in a prospective, limited multicenter (3-center) clinical observational trial [7], setting the stage for large-scale multicenter prospective efforts currently underway in the US and Europe [6,20].

Patient enrollment

Subject eligibility was based on presentation to any of the 3 Level-1 trauma centers [(Zuckerberg San Francisco General Hospital (CA), University of Pittsburgh Medical Center (PA), and University Medical Center Brackenridge (Austin, TX)] within 24 hours of injury and a history of external force trauma to the head requiring a noncontrast head CT, in accordance with the American College of Emergency Physicians/Centers for Disease Control (ACEP/CDC) criterion [21]. Patients were excluded if pregnant, in custody, non-English speaking, or on a psychiatric hold (danger to yourself or others). Between April 2010 and May 2011 the TRACK-TBI Pilot enrolled 599 acute TBI patients; 13 subjects age <16 years were excluded due to differences in variables recommended by CDE working groups, resulting in 586 subjects in the current analysis. TRACK-TBI Pilot collected 944 raw data elements per subject. From these, a set of 213 cleaned, well-curated endpoints were distilled for meaningful analysis. Eligible subjects were enrolled through convenience sampling at all three sites. Institutional Review Board (IRB) approval was obtained at all participating sites prior to study initiation. Written informed consent was obtained from all subjects prior to enrollment in the study. For patients unable to provide consent due to the severity of their injury, consent was obtained from their legally authorized representative (LAR). Patients were then re-consented, if cognitively able, at later inpatient and/or outpatient follow-up assessments for continued participation in the study. Children aged 13 and above provided their own written consent in addition to written parental/guardian consent. Clinical characteristics for patients included in the study are summarized in Table 1.

Clinical assessments and demographics

The CDE working group defined subject characteristics (i.e., demographics and social status), subject and family history, injury- or disease-related events (e.g., mechanism of injury, secondary insults), and assessments and evaluations (e.g., vital signs, intracranial pressure). The working group created a basic, intermediate, and advanced adaptation of each data element, offering investigators flexibility in the level of detail appropriate to a given study [22]. From the CDEs, the TRACK-TBI Pilot collected a combination of core, supplemental, and emerging variables.

Genetic material

DNA and acute plasma samples (<24 hours) were collected using standardized protocols developed by the NINDS TBI CDE biospecimens and biomarkers working group [23]. TRACK-TBI Pilot also followed the meticulous guidelines regarding how samples should be obtained, processed, stored locally, stored centrally, and shipped [23].

Table 1. Patient clinical characteristics.

Patient Characteristics (N = 586)	N (%)
Age (mean & standard deviation)	43.3 +/- 18.5
Sex	
Female	167 (28.5%)
Race	
White	491 (71.5%)
Education	
Below high school	68 (12.3%)
High school graduate	320 (57.7%)
Bachelor's and above	167 (30.1%)
Psychiatric History	
Present	170 (29.0%)
Previous TBI	
No	292 (52.8%)
Yes without hospitalization	103 (18.6%)
Yes with hospitalization	158 (28.6%)
Cause of Injury	
Motor vehicle accident	105 (18.0%)
MCC/bike accident	108 (18.5%)
Pedestrian hit	44 (7.5%)
Fall	199 (34.1%)
Assault	94 (16.1%)
Other	33 (5.7%)
ED admission GCS	
Severe (3–8)	42 (7.6%)
Moderate (9–12)	28 (5.1%)
Mild (13–15)	480 (87.3%)
ED admission head CT	
Positive	259 (44.2%)

Abbreviations: TBI = traumatic brain injury, MCC = motorcycle, ED = emergency department, GCS = Glasgow Coma Scale, CT = computed tomography.

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Neuroimaging

The NINDS neuroimaging working group supplied pathoanatomical definitions for 23 distinct lesion types to be used with any imaging modality. Core variables were distinguished as the presence or absence of individual lesions; lesion location and volumetric properties comprised most of the supplemental category, and emergent elements encompassed lesion-specific complexities. The imaging working group also provided recommendations for protocols to be used for both CT and MRI [24,25]. A board-certified neuroradiologist examined and coded all levels of neuroimaging variables. Recommended imaging parameters were implemented at all sites.

Outcomes

The outcomes working group delineated 12 domains of behavioral outcomes. Choosing 1 measure from 11 of the 12 domains, TRACK-TBI Pilot included a broad outcomes battery. Global outcome was assessed using a standard endpoint, the Glasgow Outcome Scale-Extended (GOS-E). The GOS-E is an 8-point clinical grading scheme for categorizing the outcome and

disability spectrum from 'dead' (GOS-E = 1), lower moderate disability (GOSE = 5), to upper good recovery (GOS-E = 8). Recovery from TBI is evidenced by achieving a higher GOS-E over time. Supplemental cognitive and psychological assessments added to a more comprehensive understanding of a domain, whereas tools in the last stages of validation were considered emerging [26]. TRACK-TBI Pilot administered the core and a subset of supplemental measures 3-, and 6-months after injury. Study personnel received *a priori* training to ensure standardization.

Topological Data Aalysis (TDA)

TDA was performed using a cloud-based analytic platform (Ayasdi, Inc. v 3.0) on 586 patients enrolled in the TRACK-TBI pilot clinical observational trial. Patients were prospectively measured on over 900 separate variables, including the NIH/NINDs common data elements (CDEs). TDA was applied to extract the fundamental outcome features across multiple clinical variables, simultaneously. For the purposes of TDA, we limited our analysis to 17 CDEs based on their clinical importance (Table 2). These 17 CDEs included CT findings, PTSD diagnosis, and cognitive measures of processing speed and verbal learning. TDA clustered patients into subgroups (nodes) based on similarity across the 17 measures, considered simultaneously as a holistic unit (Fig 1). Subgroups that share at least 1 patient in common are joined by a line (edge). The descriptive statistics of the 17 CDEs are summarized in Table 2. Missing data were only observed in the 6-month outcome variables. Determining whether there are natural subtypes within the TBI population based on these 17 CDEs presents an analytic problem that is both multi-dimensional (17 dimensions) and multi-scalar (each CDE has different range, distributional and metric features). TDA is mathematically well-suited for dealing with this complexity (see below)[8,9,11]. Simply put, TDA uses shape-based feature detection to extract the fundamental shape of the data-space. This shape is mathematically referred to as a 'reeb graph' and represents the manifold of the outcome data space. We refer to the mapping of the

Table 2. Descriptive statistics of CDE variables included in TDA to map TBI patients into a network topology based on TBI severity.

Variables used in TDA	N	Missing	Min	Max	Mean	SD
CT Brain Pathology	586	0	0	1	0.44	0.50
Skull Fracture	586	0	0	1	0.22	0.41
Skull Base Fracture	586	0	0	1	0.11	0.31
Facial Fracture	586	0	0	1	0.17	0.38
Epidural Hematoma	586	0	0	1	0.05	0.22
Subdural Hematoma	586	0	0	1	0.26	0.44
Subarachnoid Hemorrhage	586	0	0	1	0.26	0.44
Contusion	586	0	0	1	0.24	0.43
Midline Shift	586	0	0	1	0.07	0.25
Cisternal Compression	586	0	0	1	0.12	0.33
Marshall CT Score	586	0	1	6	1.76	1.10
Rotterdam CT Score	586	0	1	6	2.45	0.83
PTSD Diagnosis at 6 months (DSM-IV)	338	248	0	1	0.24	0.43
PTSD Checklist-Civilian Version at 6 months	338	248	17	83	32.98	14.80
WAIS Processing Speed at 6 months	305	281	50	150	99.20	15.96
CVLT: Short Delay Cued Recall at 6 months	296	290	-4.0	2.5	-0.08	1.14
CVLT: Long Delay Cued Recall at 6 months	295	291	-3.5	2.5	-0.19	1.17

Abbreviations: CT = computed tomography, PTSD = post-traumatic stress disorder, DSM–Diagnostic and Statistical Manual of Mental Disorders, WAIS = Wechsler Adult Intelligence Scale, CVLT = California Verbal Learning Task.

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patients within the TBI-CDEs as the 'syndromic space' of TBI (Fig 1A–1C). We refer to the TDA network as the TBI 'syndromic map' of patients within the syndromic space (Fig 1D).

TDA clustered patients using a norm correlation metric, which measures the distance between 2 points by the Pearson correlation, given by:

$$NormCorr(X, Y) = 1 - r(X', Y') \quad [1]$$

Where X', Y' are the column-wise, mean-centered, and variance normalized versions of X and Y, and

$$r(X, Y) = \frac{N \sum_{i=1}^{N} X_i Y_i - \sum_{i=1}^{N} X_i \sum_{i=1}^{N} Y_i}{\sqrt{N \sum_i X_i^2 - (\sum_i X_i)^2} \sqrt{N \sum_i Y_i^2 - (\sum_i Y_i)^2}} \quad [2]$$

This was combined with a lens called multidimensional scaling (MDS) coordinate 1 and MDS coordinate 2. These lenses generate a factorization of the data matrix into linearly uncorrelated components, with MDS coordinate 1 representing the highest variance, and MDS coordinate 2 representing the second-highest variance. The patient data are mapped into a Euclidean space, minimizing the sum of squares error, using the distance matrix rather than the coordinates. Gower's normalization is then applied prior to applying MDS to generate the lens values by:

$$f(X) - \min_z \sum_{i,j} (d(X_i, X_j) - L_2(Z_i, Z_j))^2 \quad [3]$$

TDA then resamples the MDS space millions of times in a cloud-based supercomputer, with overlapping sample bins of variable sizes to extract the shape of the data manifold. Binning size was set at a resolution of 30 and a gain of 3.0 (equalized). The resolution setting controls the number of bin partitions patients are clustered into, similar to scaling up or down on a microscope. Increasing the resolution increases the number of nodes in the analysis graph to reveal more fine structure in the syndromic space, with fewer patients per node, preserving only the strongest connections between groups of patients. Nodes that are weakly associated tend to break apart and create smaller subgroups of patients. Gain is adjusted so that most data points will appear in the same number of bins that the gain is set to. Increasing the gain increases the number of connections between nodes/groups of patients to highlight relationships within the data. Reducing the gain value will result in smaller groups of nodes and more unconnected/single nodes. Equalizing the network distributes the patients evenly across all nodes in the network.

Single Nucleotide Polymorphism (SNP) analysis

Previous bioassays from blood samples drawn from this TRACK-TBI Pilot cohort were analyzed to assess the role of specific genetic polymorphisms on patient outcome after mild TBI with targeted, hypothesis-driven analysis of 3 SNPs associated with altered striatal dopamine levels: ANKK1 C/T (rs1800497) [27], COMT Met/Val (rs4680) [28] and DRD2 C/T rs6277 [29] genotypes. These SNPs, along with 9 additional SNPs were incorporated in the TDA dataset. These newly incorporated SNPs included 2 more genes associated with striatal dopamine levels (ANKK1 C/G rs4938016, ANKK1 A/G rs11604671), the brain-derived neurotrophic factor (BDNF) gene (A/G rs6265), serotonin 5HT2A receptor (C/T rs6311), Apolipoprotein (Apo)E-ε2 (C/T rs7412) and ApoE-ε4 (C/T rs429358), mu opioid receptor OPRM1 (A/G rs1799971), B-cell lymphoma 2 (BCL2) gene (A/G rs17759659), and the Poly (ADP-ribose) polymerase (PARP-1) gene (A/T rs3219119).

Targeted hypothesis testing using General Linear Models (GLM)

SNPs found to be significantly enriched in the TDA-identified sub-groups of mild TBI patients exhibiting worse GOS-E outcome between 3 and 6 months and a positive diagnosis of PTSD, detected by the PTSD checklist, civilian version (PCL) a validated tool, were formally tested for their influence on poor outcome after TBI, including PARP1, COMT, DRD2 and the 3 different ANKK1 SNPs. The statistical model was designed as a repeated measures analysis of variance (ANOVA), testing the 3-way interaction between SNP, CT pathology (yes or no), and change in GOS-E over time (3 to 6 months) performed on the full dataset. Results are reported as both within-subject effects to tease out the influence of each polymorphism on GOS-E over time either with or without CT pathology, as well as between-subject effects to test main effects of each polymorphism on GOS-E pooled outcome across 3 and 6 months, either with or without CT pathology. This targeted hypothesis testing was performed in SPSS v.19 (IBM) using the general linear model command using type III sums-of-squares and a full factorial design. Significance was assessed at $p < .05$.

Results

Natural subtypes of TBI population as defined by CDEs in a TDA network

The generated TBI syndromic map consisted of multiple sub-networks comprising of 434 clusters (from 586 patients and 17 CDEs). Similar patients are grouped as a node (Fig 1D), with similarity defined topologically, and in a multivariate fashion from all the CDEs used in the analysis. Similar nodes are close together and joined by an edge (Fig 1E). In this way patient differences are graded by location across the syndromic map. The emergence of distinct sub-networks reflects distinct subpopulations of TBI patients. We statistically explored each sub-network to understand which CDEs play the most significant role in defining similarity and dissimilarity among patient sub-clusters.

The TBI syndromic map reveals that patients with acute pathological findings on CT (Fig 2A) and MR (Fig 2B) scans belonged to the same sub-networks, indicating that CDEs used in the analysis were able to cluster more severely injured TBI subpopulations together (red nodes on right half of network). On the other hand, the left sides of the connected sub-networks contained patients that were CT-negative and mostly MR-negative. (Fig 2A and 2B, blue clusters). The TBI syndromic map revealed relationships between patients as defined by the CDEs in a continuously-graded manner across multiple dimensions including Glasgow Coma Score (GCS), the Marshall CT score [30], Rotterdam CT score [31], and the presence of individual CT features, both categorical and quantitative. In addition, a clear CT-negative sub-network emerged with corresponding high GCS, indicating mild TBI (data not shown).

Mapping of TBI severity and long-term clinical outcome measures

Fig 2 shows the network of patients clustered on the 17 CDEs using TDA. Color schemes represent the range of values for the labeled measure, including the presence of CT positive findings (Fig 2A), MRI positive findings (Fig 2B), a positive diagnosis of PTSD according to DSM IV criteria (Fig 2C). Red nodes and connections in the network highlight positive findings for these measures, showing a clear distinction between the left (blue) and the right (red) portions of the network. Our initial observation showed that the majority of patients with a diagnosis of PTSD did not show substantial brain pathology measured by either CT or MRI. When the network was colored by the GOS-E at both 3 months (Fig 2D) and 6 months (Fig 2E) after TBI, these patients with a positive PTSD diagnosis and no obvious brain pathology (N = 19) did

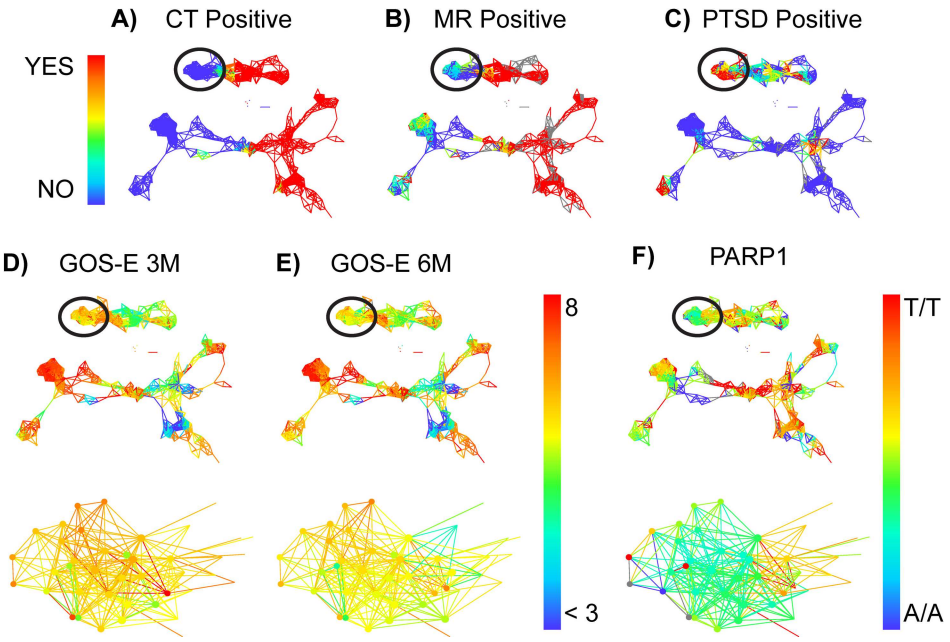

Fig 2. (A-F). TBI CDE network topology identifies the PARP1 SNP as a candidate predictor of GOS-E deficits in mild TBI. Patients with TBI were mapped into a TDA network, highlighting color schemes for CT (**A**) and MRI (**B**) pathology and whether they had a confirmed diagnosis of PTSD (DSM IV) at 6 months post-TBI (**C**). Patients in the circled regions of the network were identified due to substantial dysfunction measured by the GOS-E both at 3 months (**D**) and 6 months (**E**) post-TBI, compared with other patients in the network with no CT pathology and no diagnosis of PTSD. Data-driven exploration of these patients in the network revealed a significant categorical enrichment for the PARP1 SNP (**F**), particularly the heterozygous allele (A/T). Heat map represents range of numerical values for each measure: Panels A-C yes (1 = red) vs, no (0 = blue); Panels D-E GOS-E range from less than 3 (blue) to 8 (red); Panel F PARP1 allele A/A = 1 = blue, A/T = 2 = yellow/green, T/T = 3 = red.

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show substantial functional deficits compared to the other CT-/MR- patients (N = 43) (circled area of the network). Data-driven exploration of this region of the network revealed a significant enrichment of the PARP1 SNP (Fig 2F) measured in these patients, not previously reported by the TRACK-TBI Pilot investigators. Results from previously identified genetic polymorphisms for ANKK1 [27], COMT [28] and DRD2 [29] were confirmed to have an impact on outcome deficits in patients with TBI (Figures in S1–S3 Figs, Tables in S1–S6 Tables).

In order to formally test the hypothesis that the PARP1 SNP was a significant predictor of GOS-E recovery in patients with mild TBI, we performed an independent analysis on the full dataset using a 3-way mixed general linear model with repeated measures. This analysis was structured as a balanced factorial design testing the impact of the following factors on GOS-E recovery: Time (repeated measure; 3 vs. 6 months), CT findings (between-subjects; yes/no) and PARP1 genotype (between subjects: AA, AT, TT). Significant between-subject effects were detected in the 3-way analysis: time by CT by PARP1 genotype interaction (N = 122 patients, PARP1 A/A (n = 33), A/T (n = 44), T/T (n = 45), p = 0.019). Patients with the T/T and A/T genotypes performed worse over time on the GOS-E compared with patients with the A/A genotype in the patients with no CT pathology (Fig 3, Tables 3 and 4). Clinical characteristics of patients in the TDA-selected subgroup circled in Fig 2 (N = 37) are summarized in Table 5, alongside clinical characteristics for all patients with data collected and analyzed for the PARP1 SNP (N = 298). The TDA-selected patient group was slightly younger (41.1 ± 14.2 TDA group, vs 43.5 ± 18.2 all PARP1 group), with 6.1% fewer females, 22.4% fewer Caucasians, and roughly

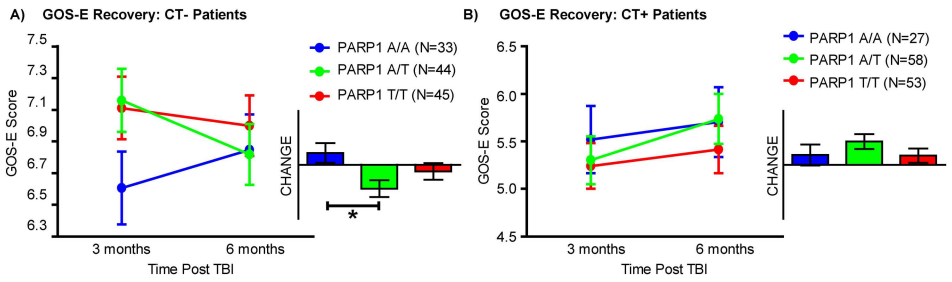

Fig 3. (A-B). Hypothesis testing of PARP1 genetic polymorphism influence on GOS-E deficits in mild TBI. GOS-E scores between 3 and 6 months post-TBI were plotted for patients who were CT negative (**A**) or CT positive (**B**), based on the SNP allele expressed (A/A = blue, A/T = yellow/green, T/T = red). Hypothesis testing of the interaction between CT pathology and the SNP allele over time revealed a significant 3-way interaction; however, no significance was detected at each time point individually. Only change in GOS-E over time was significant in patients with a negative head CT.*p < .05.

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6% less likely to have finished high school or college. TDA selected patients also had 15% less previous psychiatric history, however were more likely to have a previous TBI, either with (28.9%) or without hospitalization (9.2%), and were 22% more likely to have received their TBI from an assault.

Hypothesis testing of the interaction between CT pathology and the ANKK1 SNP allele on GOS-E outcome over time revealed a significant 3-way interaction for ANKK1 Gly422Arg (rs4938016) only, and a significant difference in GOS-E scores at both 3 and 6 months for patients with a positive head CT for ANKK1 Gly318Arg (rs11604671). However, these differences were not found to significantly change over time (Figure in S1 Fig, Tables in S1 and S2 Tables). Hypothesis testing of the interaction between CT pathology and the COMT SNP allele on GOS-E outcome over time revealed both a significant influence of COMT on GOS-E recovery over time, and a 3-way interaction of GOS-E recovery time with the SNP allele and presence/absence of CT pathology, specifically in patients with a negative head CT (Figure in S2 Fig, Tables in S3 and S4 Tables). Hypothesis testing of the interaction between CT pathology and the DRD2 SNP allele on GOS-E outcome over time revealed a significant influence of DRD2 on GOS-E at 3- and 6-months post TBI; however, this was only detected in patients with a positive head CT and did not significantly change over time (Figure in S3 Fig, Tables in S5 and S6 Tables).

Table 3. General linear model statistics for PARP1 SNP interaction with CT pathology on GOS-E recovery.

CT Pathology x SNP Interactions															
Source	**GOSE Score (3M)**					**GOSE Score (6M)**					**GOSE Score (3M to 6M Change)**				
	SS	**df**	**MS**	**F**	**Sig.**	**SS**	**df**	**MS**	**F**	**Sig.**	**SS**	**df**	**MS**	**F**	**Sig.**
PARP1 (rs3219119)	.19	2	.09	.03	.97	3.16	2	1.58	.45	.64	1.80	2	.90	.81	.45
CT Pathology x PARP1 (rs3219119)	3.66	2	1.83	.54	.58	11.00	2	5.50	1.57	.21	8.94	2	4.47	4.03	***.02**
Multiple Comparisons (Tukey HSD posthoc test)	A/A vs A/T				NT	A/A vs A/T				NT	A/A vs A/T				0.47
	A/A vs T/T				NT	A/A vs T/T				NT	A/A vs T/T				0.57
	A/T vs A/A				NT	A/T vs A/A				NT	A/T vs A/A				0.47
	A/T vs T/T				NT	A/T vs T/T				NT	A/T vs T/T				0.98
	T/T vs A/A				NT	T/T vs A/A				NT	T/T vs A/A				0.57
	T/T vs A/T				NT	T/T vs A/T				NT	T/T vs A/T				0.98

Abbreviations: SS = Type III Sum of Squares, df = degrees of freedom, MS = mean square, NT = not tested,
* = statistical significance.

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Table 4. General linear model statistics for PARP1 SNP interaction on GOS-E recovery by presence or absence of CT pathology.

CT Negative																CT Positive													
Source	GOSE Score (3M)					GOSE Score (6M)					GOSE Score (3M to 6M Change)					Source	GOSE Score (3M)					GOSE Score (6M)					GOSE Score (3M to 6M Change)				
	SS	df	MS	F	Sig.	SS	df	MS	F	Sig.	SS	df	MS	F	Sig.		SS	df	MS	F	Sig.	SS	df	MS	F	Sig.	SS	df	MS	F	Sig.
PARP1 (rs3219119)	2.38	2	1.19	.54	.58	7.64	2	3.82	1.65	.20	9.24	2	4.62	3.84	***0.02**	PARP1 (rs3219119)	1.44	2	.72	.16	.85	6.76	2	3.38	.71	.50	2.17	2	1.08	1.07	.35
Multiple Comparisons (Tukey HSD posthoc test)	A/A vs A/T				NT	A/A vs A/T				NT	A/A vs A/T				***0.02**	Multiple Comparisons (Tukey HSD posthoc test)	A/A vs A/T				NT	A/A vs A/T				NT	A/A vs A/T				NT
	A/A vs T/T				NT	A/A vs T/T				NT	A/A vs T/T				0.33		A/A vs T/T				NT	A/A vs T/T				NT	A/A vs T/T				NT
	A/T vs A/A				NT	A/T vs A/A				NT	A/T vs A/A				***0.02**		A/T vs A/A				NT	A/T vs A/A				NT	A/T vs A/A				NT
	A/T vs T/T				NT	A/T vs T/T				NT	A/T vs T/T				0.31		A/T vs T/T				NT	A/T vs T/T				NT	A/T vs T/T				NT
	T/T vs A/A				NT	T/T vs A/A				NT	T/T vs A/A				0.33		T/T vs A/A				NT	T/T vs A/A				NT	T/T vs A/A				NT
	T/T vs A/T				NT	T/T vs A/T				NT	T/T vs A/T				0.31		T/T vs A/T				NT	T/T vs A/T				NT	T/T vs A/T				NT

Abbreviations: SS = Type III Sum of Squares, df = degrees of freedom, MS = mean square, NT = not tested,

* = statistical significance.

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Table 5. Clinical characteristics of TDA-identified patient subgroup with PARP1 SNP (N = 37) alongside to all patients with PARP1 SNP (N = 298).

Patient Characteristics	All PARP1 Patients (N = 298)	TDA Subgroup (N = 37)
Age (mean & standard deviation)	43.5 +/- 18.2	41.1 +/- 14.2
Sex		
Female	91 (30.5%)	9 (24.4%)
Race		
White	252 (84.6%)	23 (62.2%)
Education		
Below high school	27 (9.1%)	9 (25%)
High school graduate	167 (56.0%)	18 (50%)
Bachelor's and above	93 (31.2%)	9 (25%)
Psychiatric History		
Present	93 (31.2%)	6 (16.2%)
Previous TBI		
No	152 (51.0%)	6 (16.2%)
Yes without hospitalization	53 (17.8%)	10 (27.0%)
Yes with hospitalization	83 (27.9%)	21 (56.8%)
Cause of Injury		
Motor vehicle accident	50 (16.8%)	3 (8.1%)
MCC/bike accident	55 (18.5%)	5 (13.5%)
Pedestrian hit	24 (6.0%)	2 (5.4%)
Fall	107 (35.9%)	11 (29.7%)
Assault	47 (15.8%)	14 (37.8%)
Other	14 (4.7%)	2 (5.4%)
ED admission GCS		
Severe (3–8)	26 (8.7%)	0 (0%)
Moderate (9–12)	13 (4.4%)	0 (0%)
Mild (13–15)	230 (77.2%)	37 (100%)
ED admission head CT		
Positive	144 (48.3%)	0 (0%)
PARP1 SNP		
A/A	67 (22.5%)	9 (37.5%)
A/T	116 (38.9%)	9 (37.5%)
T/T	115 (38.6%)	6 (25%)

Abbreviations: PARP1 = Poly [ADP-ribose] polymerase 1, TDA = topological data analysis, TBI = traumatic brain injury, MCC = motorcycle, ED = emergency department, GCS = Glasgow Coma Scale, CT = computed tomography, SNP = single nucleotide polymorphism.

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TDA uncovered a subgroup of mild TBI individuals with poorer outcome, associated with increased PTSD rates and specific single-nucleotide polymorphisms (SNPs) associated with DNA damage and brain dopamine processing. The results provide proof-of-concept for application of multi-scalar big-data analytics to improve TBI precision medicine

Discussion

TDA applied to data from multiple CT and MR imaging and neuropsychological domains captured the multidimensional locus of individual patients within the TBI syndromic space. Rapid mapping of TBI outcome onto the TDA-syndromic space revealed that mild TBI can be

stratified into multiple subgroups that have differentiated outcome. A large subpopulation of mild TBI subjects showed poor recovery and tendency to deteriorate from 3–6 months post-injury (Fig 2D and 2E). These same individuals had very high rates of PTSD (Fig 2C) and significant enrichment in the heterozygous allele of the PARP1 SNPs (Fig 2F) that is associated with cellular responses to stress and DNA damage [32,33].

TDA improves upon traditional outcome-prediction approaches for TBI that have relied on regression modeling of multiple predictors with respect to a single 'gold-standard' outcome measure (e.g., the GOS-E). By simultaneously leveraging the full information provided by all outcomes, TDA and related big-data approaches have potential to improve diagnosis and therapeutic targeting. For example, CT features and neuropsychiatric batteries provided alternative views of injury severity within the topological syndromic map (Fig 2), and considering each of these pieces of information in isolation would provide only a limited view of the full syndrome of TBI. Therefore, once the TBI syndromic space was established using the pre-selected CDEs (Table 2), we were able to harness this full set of information for all patients to discover novel predictors of recovery following TBI, including several SNPs. The most striking genetic biomarker finding was that PARP1 predicted recovery in patients with a negative head CT, who would be considered to have a mild TBI (mTBI). Previous studies have implicated PARP1 as a useful therapeutic target in humans with TBI, particularly in patients with severe TBI that are enriched for A/A allele [32]. Additionally, attempts to inhibit PARP1 in rat models of TBI have shown promise in helping to reduce cell death [33]. Therefore, PARP1 may be a useful biomarker in mTBI patients when considering patient trajectories and how to maximize recovery in patients presenting with this particular A/T SNP (rs3219119) of the PARP1 gene.

TDA also confirmed the influence of genes involved in dopamine processing reported previously in TRACK-TBI Pilot patients for ANKK1 [27] (Figure in S1 Fig, Tables in S1 and S2 Tables) and COMT [28] (Figure in S2 Fig, Tables S3 and S4 Tables), as well as the novel findings of an influence of the DRD2 SNP C/C allele associated with better recovery of GOS-E in patients with a positive head CT (Figure in S3 Fig, Tables in S5 and S6 Tables), however recent findings have suggested that the T/T allele may be predictive of better recovery on verbal learning tasks after correcting for injury severity [29]. These genes represent divergent molecular mechanisms that result in lowered brain dopamine signaling. ANKK1 T/T is associated with a 40% reduction in the DRD2 receptor [34], whereas the rs4680 SNP encodes for the Met158Val locus of COMT, and the G/G genotype has been associated with lower dopamine levels due to the increase in enzymatic activity [35]. Previous studies have investigated the effect of this mutation on personality traits, dubbing the resulting phenotype as "warrior" compared to its "worrier" counterpart. The "warrior" phenotype is associated with higher concentration, memory, and cognitive function with mixed reports on the ability to emotionally process stimuli. Specifically, there have been multiple studies linking the rs4680 G/G genotype with schizophrenia [36] and lower drug responsiveness for antidepressants and anti-narcoleptics [37,38]. The association of TBI outcome to these genotypes may be due to decreased dopamine levels rather than the specific biomolecular mechanism, leaving still unanswered questions regarding the inherent predisposition to outcome and drug responsiveness of individuals suffering traumatic brain injuries.

Taken together the results indicate that, COMT and PARP1 may be useful biomarkers in a clinical prediction model to determine whether patients with an initial diagnosis of a mild TBI will develop significant functional deficit as measured on the GOS-E. ANKK1 and DRD2, on the other hand, may be useful biomarkers in a clinical prediction model for severe TBI, and warrants further investigation and cross-validation in a larger patient cohort to test whether mitigating the downstream effects of these genetic variants will improve outcome following TBI.

The present findings illustrate the value of TDA for expanding upon traditional diagnostic and prognostic tools for TBI. TDA exhibits several benefits as compared with regression methods, which perform poorly with numerous inter-correlated (multi-collinear) variables. In a regression context, multi-collinearity can lead to over-fitting to a particular dataset, limiting diagnostic value for distinct patient populations. In addition, traditional multiple regression models for TBI have been constructed to explain the variance of a single 'gold standard outcome,' for example the GOS-E. Such approaches ignore the fact that TBI outcome is intrinsically multifaceted. The most precise patient information is captured by considering all of the domains (e.g., psychological, cognitive) of outcome simultaneously, as is possible with TDA. Finally, traditional statistical approaches are designed to maximize the variance explained (predicted) in outcome and their performance is benchmarked by assessing value added over alternative/competing models. TDA does not suffer from these limitations because it is fundamentally focused on extracting the most robust shape (persistent homology) [8,9] across multiple alternative data views through numerous dimensions, different patient clustering algorithms, and patient subpopulations. In essence, TDA provides direct visualization of the shape of multidimensional TBI, enabling rapid insight-discovery not achievable through traditional analytics.

TDA and similar integrative analytics hold great promise to further propel recent advances in the use of novel molecular biomarkers, imaging biomarkers, and psychosocial outcomes for TBI [6,7,39–41]. To develop targeted therapeutic interventions, TBI clinician-researchers face the complex task of stratifying patients based on multifaceted information, and integrating information about TBI is fundamentally a data-intensive undertaking that could benefit from the application of advanced statistical pattern-detection approaches for enhanced decision support. Through integrative analytics of TRACK-TBI Pilot and similar datasets from other CNS diseases, TDA may help realize the potential of precision medicine to rapidly and accurately classify TBI and to identify subpopulations to target with precision medicine approaches.

Supporting information

S1 Fig. ANKK1 SNP distribution in TDA network and hypothesis testing on GOS-E recovery between 3 and 6 months post-TBI. (**A**) Distribution of 3 separate ANKK1 SNPs in the TDA network. (**B**) GOS-E scores between 3 and 6 months post-TBI were plotted for patients who were either CT negative or CT positive, grouped based on the SNP allele expressed. Hypothesis testing of the interaction between CT pathology and the ANKK1 SNP allele on GOS-E outcome over time revealed a significant 3-way interaction for ANKK1 Gly422Arg (rs4938016) only, and a significant difference in GOS-E scores at both 3 and 6 months for patients with a positive head CT for ANKK1 Gly318Arg (rs11604671). However, these differences were not found to significantly change over time. $^{*}p < .05$.
(TIF)

S2 Fig. COMT SNP distribution in TDA network and hypothesis testing for impact on GOS-E recovery between 3 and 6 months post-TBI. (**A**) Distribution of the COMT SNP in the TDA network. (**B**) GOS-E scores at 3 and 6 months post-TBI were plotted for patients who were CT negative or CT positive, group based on the SNP allele expressed (Met/Met = blue, Met/Val = yellow/green, Val/Val = red). Hypothesis testing of the interaction between CT pathology and the COMT SNP allele on GOS-E outcome over time revealed both a significant association of COMT with GOS-E recovery over time, and a 3-way interaction of GOS-E recovery with the SNP allele and presence/absence of CT pathology, specifically in patients with negative head CT. # $p < .05$ compared to both groups.
(TIF)

S3 Fig. DRD2 SNP distribution in TDA network and hypothesis testing on GOS-E recovery between 3 and 6 months post-TBI. (**A**) Distribution of the DRD2 SNP in the TDA network. (**B**) GOS-E scores between 3 and 6 months post-TBI were plotted for patients who were CT negative or CT positive, group based on the SNP allele expressed (C/C = blue, C/T = yellow/green, T/T = red). Hypothesis testing of the interaction between CT pathology and the DRD2 SNP allele on GOS-E recovery revealed a significant association of DRD2 with GOS-E at 3 and 6 months post TBI, however this was only detected in patients with a positive head CT and did not significantly change over time. $^{*}p < .05$.
(TIF)

S1 Table. General linear model statistics for ANKK1 SNP interaction with CT pathology on GOS-E recovery.
(DOCX)

S2 Table. General linear model statistics for ANKK1 SNP interaction on GOS-E recovery by presence or absence of CT pathology.
(DOCX)

S3 Table. General linear model statistics for COMT SNP interaction with CT pathology on GOS-E recovery.
(DOCX)

S4 Table. General linear model statistics for COMT SNP interaction on GOS-E recovery by presence or absence of CT pathology.
(DOCX)

S5 Table. General linear model statistics for DRD2 SNP interaction with CT pathology on GOS-E recovery.
(DOCX)

S6 Table. General linear model statistics for DRD2 SNP interaction on GOS-E recovery by presence or absence of CT pathology.
(DOCX)

S1 Dataset. Minimal dataset of variables used to generate and color the TDA network. Variables included in this minimal dataset are those described in Table 2 as well as GOS-E and selected SNPs for PARP1, ANKK1, COMT and DRD2 used for hypothesis testing. The first column of the dataset is the global unique identifier for the TRACK-TBI pilot dataset, which can be used to link to additional variables from these patients in the full dataset stored in the Federal Interagency Traumatic Brain Injury Research (FITBIR) informatics system (https://fitbir.nih.gov/) and the One Mind Portal (http://onemind.org/Our-Solutions/One-Mind-Portal). Access to the full dataset can be requested by qualified researchers through these data portals.
(XLSX)

S1 Metadata. Relevant metadata for S1 Dataset to understand description and value ranges and codes for each variable used to generate and color the TDA network. Variables listed in column A of the S1 Metadata file are copied and transposed from the first row of variables in the S1 Dataset, and accompanied by definitions and value ranges and ordinal codes for each variable.
(XLSX)

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Resting-State Functional Connectivity Alterations Associated with Six-Month Outcomes in Mild Traumatic Brain Injury

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Abstract

Brain lesions are subtle or absent in most patients with mild traumatic brain injury (mTBI) and the standard clinical criteria are not reliable for predicting long-term outcome. This study investigates resting-state functional MRI (rsfMRI) to assess semiacute alterations in brain connectivity and its relationship with outcome measures assessed 6 months after injury. Seventy-five mTBI patients were recruited as part of the prospective multicenter Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) pilot study and compared with matched 47 healthy subjects. Patients were classified following radiological criteria: CT/MRI positive, evidence of lesions; CT/MRI negative, without evidence of brain lesions. rsfMRI data were acquired and then processed using probabilistic independent component analysis. We compared the functional connectivity of the resting-state networks (RSNs) between patients and controls, as well as group differences in the interactions between RSNs, and related both to cognitive and behavioral performance at 6 months post-injury. Alterations were found in the spatial maps of the RSNs between mTBI patients and healthy controls in networks involved in behavioral and cognition processes. These alterations were predictive of mTBI patients' outcomes at 6 months post-injury. Moreover, different patterns of reduced network interactions were found between the CT/MRI positive and CT/MRI negative patients and the control group. These rsfMRI results demonstrate that even mTBI patients not showing brain lesions on conventional CT/MRI scans can have alterations of functional connectivity at the semiacute stage that help explain their outcomes. These results suggest rsfMRI as a sensitive biomarker both for early diagnosis and for prediction of the cognitive and behavioral performance of these patients.

Keywords: cognitive and behavioral outcome; rsfMRI; TBI

Introduction

Symptoms after mild traumatic brain injury (mTBI) may be somatic, cognitive, or psychiatric, and although it is often assumed that there will be total recovery within the first 3 months after an episode of mTBI, in some patients symptoms may be persistent and may result in lifelong disability.[1] The diagnosis and prognosis of mTBI continue to be a challenge, and misdiagnosis is common.[2,3] Symptomatology and clinical neuroimaging are not sufficiently sensitive to allow the detection of subtle brain changes that occur after mTBI. These changes may be the cause of persistent postconcussive symptoms and cognitive/behavioral impairments. Therefore, it is extremely important to find biomarkers capable of diagnosing changes in the brain that occur after mTBI, permitting the identification of patients who will require specific short- and long-term therapeutic interventions.

Through the analysis of temporal correlations of the blood-oxygenation-level- dependent (BOLD) signal in different gray matter regions, resting-state functional MRI (rsfMRI) allows the noninvasive study of brain networks and their interactions. The

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main resting-state networks (RSNs) are well characterized,[4] and maps of functional connectivity within RSNs have been shown to match patterns of task-related increases in brain activity associated with a variety of cognitive and behavioral domains.[5] Abnormal functional connectivity has been reported in mTBI patients in the default mode network (DMN),[6–10] which consists of a set of the brain regions that remain active while the brain is at rest, and that deactivate when there are external behavioral demands.[11] Although the default mode network is the most widely studied RSN, other networks and regions, such as the thalamic network,[12,13] or the frontoparietal and motor-striatal networks,[14] have also been reported as being disrupted after mTBI. Stevens and coworkers[15] also found abnormal increases and decreases in the connectivity of numerous networks in addition to the DMN. Although most studies have focused on the status of specific networks in isolation, it is important also to address how RSNs interact with one another to give efficient responses to environmental stimuli.

To date, rsfMRI studies of mTBI have been limited by factors such as small sample size, wide spectrum of injury severity, large variation in time point after injury ranging from acute to chronic, and variability in the clinical criteria used for evaluating patients.[16] There have also been relatively few data published on the correlation of early rsfMRI changes with long-term outcome in mTBI. In this study, we hypothesize that altered functional connectivity within and between RSNs at the semi-acute stage, $\sim$1–2 weeks after mTBI, will be related to postconcussive symptoms and to cognitive deficits 6 months after injury.

Methods

Participants

A sample of 75 mTBI patients recruited at San Francisco General Hospital (SFGH) as part of the prospective multi-center Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) pilot study[17] was included in this investigation. The detailed characteristics of this sample have been described in detail elsewhere.[16] The inclusion criteria included CT scan to assess for evidence of acute TBI within 24 h of injury, Glasgow Coma Scale (GCS) score 13–15 (upon emergency department [ED] arrival), loss of consciousness (LOC) <30 min, post-traumatic amnesia (PTA) duration <24 h, and age 18–55 years (inclusive). Exclusion criteria were: lack of fluency in English, contraindication for MRI, and a reported history of previous TBI resulting in LOC >5 min. Four of the subjects were excluded because of artifacts in the rsfMRI data.

CT was performed within 2–3 h of TBI. MRI was performed within 11.2 ± 3.3 days (range, 5–18) post-injury. A 7 min. rsfMRI single shot gradient-echo echo planar imaging (EPI) sequence was acquired (repetition time [TR] = 2000 ms, echo time [TE] = 28 ms; flip angle = 90 grad; field of view [FOV] = 220 mm; voxel size = 3.4 × 3.4 × 4.0 mm). The subjects were asked to close their eyes, relax, not focus their attention on anything specific, and not fall asleep. All CT examinations were performed on a GE Lightspeed 64-row-detector CT scanner, and all MRIs were performed on the same 3T GE Signa EXCITE scanner equipped with an eight channel phased array head radiofrequency coil (GE Healthcare, Waukesha, WI), using the same scanner software version. The following conventional 3T MRI sequences were performed: 1) axial three-dimensional (3D) inversion recovery fast spoiled gradient recalled echo T1-weighted images (TE = 1.5 ms; TR = 6.3 ms; inversion time [TI] = 400 ms; flip angle, 15 degrees) with 230 mm FOV, 156 contiguous partitions (1.0 mm) at 256 × 256 matrix; 2) axial T2-weighted fluid-attenuated inversion recovery images (TE = 126 ms; TR = 10 sec; TI = 2200 ms) with 220 mm FOV, 47–48 contiguous slices (3.0 mm) at 256 × 256 matrix; and 3) axial magnetization-prepared gradient echo T2*- weighted images (TE = 15 ms; TR = 500 ms; flip angle 20 degrees) with 220 × 170 mm FOV and 47–48 contiguous slices (3.0 mm) at 256 × 192 matrix. The MRI scanner and the scanning protocol used were the same for the group of patients and for the healthy control group.

Each patient's head CT upon ED presentation and semiacute brain MRI were characterized using the TBI common data elements (TBI-CDE).[17] Each CT and MRI was anonymized and reviewed by a board certified neuroradiologist blinded to the data. The mTBI patients were divided into two subgroups: 1) CT/MRI positive ($n = 31$; age: $\bar{x} = 34 \pm 12.2$ years), defined as patients with any acute traumatic intracranial lesion (epidural hematoma [EDH], subdural

Table 1. MRI Radiological Findings of the CT/MRI Positive TBI Group

1	2 microhemorrhages (R fr opercular)
2	5–7 microhemorrhages (L sup fr gyr, L sup parietal, L splenium, L genu, R PLIC); small R/L SDH
3	2 microhemorrhages
4	1 microhemorrhage (L temp)
5	5–7 microhemorrhages (L/Rmid-inf temp gyr, R inf fr gyr, genu); contusions (R middle fr gyr, R parietal) R/L fr EDH. SDH; R fr-parietal skull fractures
6	Contusions (L sup fr gyr, R sup fr gyr, R/L ant orbit, R inf temp gyr, L med temp; SDHs
7	2 microhemorrhages (L sup fr gyr, L cing)
8	2 microhemorrhages (R rostrum/genu, L sup parietal); L fr-temp EDH; contusions (R fusiform gyr, R mid temp gyr, R inf temp gyr); SDH.
9	1 microhemorrhage (L cingulum single focus); L med orb gyr encephalomalacia
10	3 microhemorrhages (R genu, R frontal horn, R subinsular WM)
11	1 microhemorrhage (R fr hem shear); contusions (R med orb, L inf fr gyr, R inf temp gyr, L ant temp gyr)
12	Contusions (R mid and inf temp); SDH
13	3 microhemorrhages (R mid fr gyr, L postcentral gyr, L parietal); contusion (R sup temp gyr); SDH
14	1 microhemorrhage (R frontal shear)
15	2 microhemorrhages L fr subcortical white matter
16	2 microhemorrhages (R post limb of internal capsule)
17	1 microhemorrhage (post L temp WM)
18	2 microhemorrhages (R periventricular); contusion (R medial orbital)
19	2 microhemorrhages (L & R CGH); contusions (L sup, mid, inf temp gyr, L fr opercular)
20	2 microhemorrhages (L CGH, L sup fr gyr)
21	3 microhemorrhages (R genu, L sup fr gyr)
22	2 microhemorrhages (L post temp, R postcentral gyr); contusions (L mid- inf fr gyr, L sup- mid temp gyr)
23	3 microhemorrhages (B ant temp and R occ WM); contusions (R frontal, B occ contusions)
24	2 microhemorrhages (R CGH, L post temp WM)
25	2 microhemorrhages (L precentral gyr, L sup fr gyr)
26	4 microhemorrhages (L sup fr gyr, R fr operculum)
27	2 microhemorrhages (L ant and post temp WM)
28	1 microhemorrhage (R ant temp)
29	2 microhemorrhages (L sup parietal lobule)
30	2 microhemorrhages (L and R ant temp WM)
31	Small L SDH

TBI, traumatic brain injury; PLIC, posterior limb of the internal capsule; CGH, cingulum hippocampal gyrus; SDH, subdural hematoma; L, left; R, right; B, bilateral, EDH, epidural hematoma; WM, white matter; sup, superior; mid, middle; inf, inferior; ant, anterior; post, posterior; fr, frontal; temp, temporal; occ, occipital; gyr, gyrus; orb, orbital.

hematoma [SDH], subarachnoid hemorrhage [SAH], contusion, or evidence of traumatic axonal injury[TAI]) and/or depressed skull fracture on either CT or MRI, and 2) CT/MRI negative ($n=44$; age: $\bar{x}=31\pm9.5$ years), defined as patients without any such abnormality on either CT or MRI.[14] The radiological MRI findings of the CT/MRI positive TBI group are displayed in Table 1. The age group comparisons between mTBI groups were not statistically significant ($n=75$; $\bar{x}=32.36\pm10.7$ years, $p=0.28$). The shapes of the age distributions of the two groups were also not statistically significant, as measured by Kolmogórov–Smirnov test (K-S) ($p=0.60$). The patients' GCS scores ranged from 13 to 15 (mTBI positive GCS [15/14/13]=19/11/1; mTBI negative GCS [15/14/13]=36/7/1).

The outcome measures included the Extended Glasgow Outcome Scale (GOS-E) at 6 months post-injury performed through structured interviews with each participant by research assistants trained to uniformly assess the GOS-E. A trained neuropsychologist administered the following behavioral and cognitive tests to the mTBI patients 6 months after injury: the Rivermead Postconcussion Symptoms Questionnaire (RPQ) consisting of 16 physical and psychosocial symptoms frequently reported after mTBI, the California Verbal Learning Test–Second Edition (CVLT-II) to evaluate learning, short and long-term memory, and the Trail Making Tests (TMT) A and B to evaluate attention, processing speed, and cognitive flexibility to switch tasks as well as executive function. No cognitive testing data were available for the control group.

The control group consisted of 47 healthy subjects matched with the patients group by age ($\bar{x}=28.8\pm9$ years; ANOVA: $F=2.5$ $p=0.08$) and education (ANOVA: $F=2.5$ $p=0.08$) without previous diagnosis of TBI, or neurological or psychiatric disorders. The shape of the age distributions measured by K-S was not significant between the control group and either the mTBI CT/MRI positive or the mTBI CT/MRI negative group ($p=0.30$ and $p=0.40$, respectively).

Statistical analysis

The summary of the imaging data preprocessing and analysis is shown in Figure 1.

Resting-state fMRI data were first preprocessed and then analyzed using probabilistic independent component analysis (ICA), implemented in MELODIC, followed by a network-based approach with FSLNets, a toolbox for performing basic network modelling from fMRI time series data. All procedures are part of FSL (http://fsl.fmrib.ox.ac.uk/fsl).

First we performed standard preprocessing of resting-state fMRI data, which included brain extraction,[18] motion correction,[19] and spatial smoothing using a Gaussian kernel with a full-width at half maximum (FWHM) of 6 mm and high-pass temporal filtering with a 100 sec cutoff. Functional scans were then registered to each subject's high-resolution MPRAGE scan using affine linear registration (FMRIB's Linear Image Registration Tool [FLIRT]) and further registered to the common Montreal Neurological Institute (MNI) standard space using linear affine registration with 12 degrees of freedom.

We then used ICA-based Xnoiseifier artifact removal (FIX) to de-noise single subject data.[20] For this, we performed a single-session ICA and the resulting components were introduced into FIX, which identified the ''bad'' components and removed them from the individual preprocessed fMRI timeseries. Fifteen subjects were selected to create a training data set to classify the ICA components into ''good'' or ''bad.'' Then, in order to obtain the study group maps, a group-level ICA was performed in the new ''clean'' data using temporal concatenation of fMRI data from all the subjects, and restricted to 25 independent components (ICs).

A dual regression approach[21] was then used to find between-group differences in the connectivity maps for each component. The group-ICA maps were first regressed against each individual

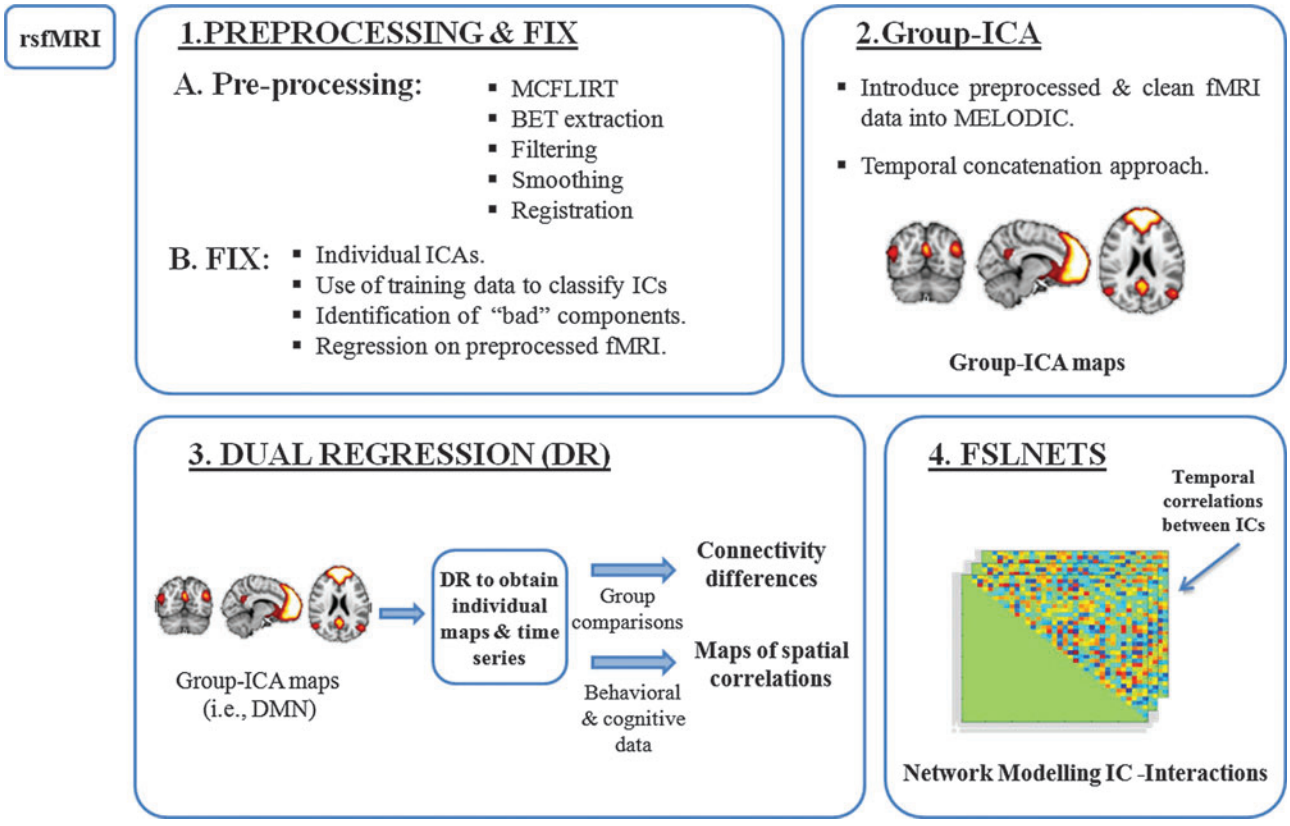

FIG. 1. Imaging data preprocessing and analysis summary. Color image is available online at www.liebertpub.com/neu

preprocessed fMRI series (spatial regression) to obtain sets of time series that are specific to each subject and each IC. At a second stage, these time series were regressed again to the individual fMRI data (temporal regression) to obtain IC Z-maps specific for each subject and each component. Finally, these individual maps were compared between subjects using a voxelwise general linear model (GLM) analysis with permutation testing to correct for multiple comparisons[22] using threshold-free cluster enhancement (TFCE) family-wise error corrected (FWE) corrected at $p \leq 0.05$. Further, to assess resting-state network interactions, whole brain

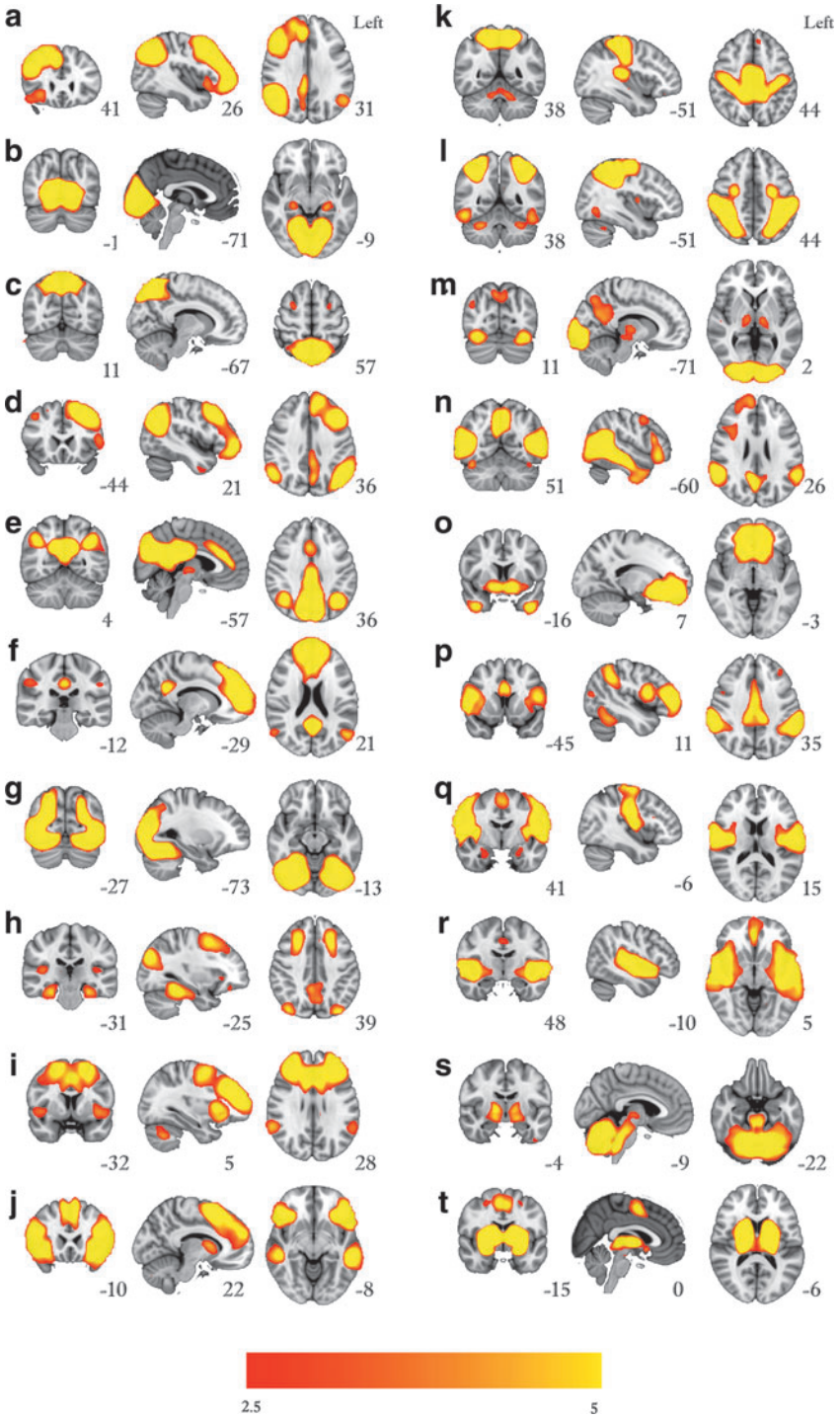

FIG. 2. Resting-state networks (RSNs) from the independent component analysis (ICA) group decomposition. **(a)** Frontoparietal right network; **(b)** primary visual network; **(c)** superior parietal network; **(d)** frontoparietal left; **(e)** default mode network (DMN) posterior part; **(f)** DMN; **(g)** occipito-cerebellar network; **(h)** ventral attentional network; **(i)** executive control network; **(j)** salience network; **(k)** upper somatomotor network; **(l)** dorsal attentional network; **(m)** visual network; **(n)** dorsal and ventral visual stream; **(o)** orbitofrontal network; **(p)** cingular opercular network; **(q)** lower somatomotor network; **(r)** auditory network**; (s)** brainstem and cerebellum network; **(t)** basal ganglia. Color image is available online at www.liebertpub.com/neu

connectivity matrices were created from the individual network time series with FSLNETs[23] (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). In these matrices, nodes were defined from the group ICA maps. Time series were then obtained by the spatial regression of these maps to the preprocessed 4D data sets (i.e., first stage of the dual regression procedure). Because of the nature of ICA maps, each node would be a map covering the whole brain with the strongest weight of the regions showing higher Z-scores in that specific map. After defining the nodes and their associated time series, each edge represents the connectivity between pairs of nodes, computed using full correlation. These matrices were then analyzed in a group-level approach, and GLM was used to find group differences and correlations with cognitive and clinical outcomes. Results of the RSNs interactions were corrected for multiple comparisons using the false discovery rate (FDR).

Results

Cognitive and behavioral data

No significant differences were found in any of the cognitive and behavioral data between the CT/MRI mTBI positive and CT/MRI mTBI negative groups.[16]

RSN spatial maps

We identified 20 RSNs from the group ICA decomposition (Fig. 2) by visual inspection and using templates available in the literature.[4] The detailed description of the networks is described in Table S1 (see online supplementary material at http://www.liebertpub.com).

We performed four group RSN spatial maps comparisons analysis: 1) mTBI CT/MRI positive and negative ($n=75$) versus healthy control group; 2) CT/MRI mTBI positive ($n=31$) versus healthy control group; 3) CT/MRI mTBI negative ($n=44$) versus healthy controls; and 4) mTBI CT/MRI positive group ($n=31$) versus CT/MRI mTBI negative group ($n=44$).

We found significant differences in connectivity within the spatial patterns of the main RSNs for the mTBI patient group as a whole when compared with the control group (Fig. 3). mTBI patients showed reduced connectivity in the frontal nodes of the DMN, executive control network, frontal nodes of the frontoparietal network (FP-right), parietal areas of the dorsal attentional network, and the frontal node of the orbitofrontal network, together with an increase in the connectivity of the visual network.

We also found significant differences for each of the two subgroups of mTBI patients compared with the healthy controls (Fig. 4). mTBI patients with CT/MRI positive scans showed reductions in connectivity in frontal brain areas in the same abovementioned RSNs, whereas the mTBI patients with CT/MRI negative scans showed reduced connectivity in the orbitofrontal network and the DMN and, additionally, demonstrated reductions in the salience network and an increase in the connectivity of the visual network. No significant differences in connectivity were found when comparing the RSN spatial maps between the CT/MRI mTBI positive versus the CT/MRI mTBI negative subgroups.

All results were corrected for multiple comparisons by using FWE correction at $p<0.05$.

Correlations of RSNs with 6months postconcussive symptoms

Negative correlations between semiacute connectivity and RPQ score at 6 months were found within the posterior regions of several networks only in the CT/MRI negative group (Fig. 5).

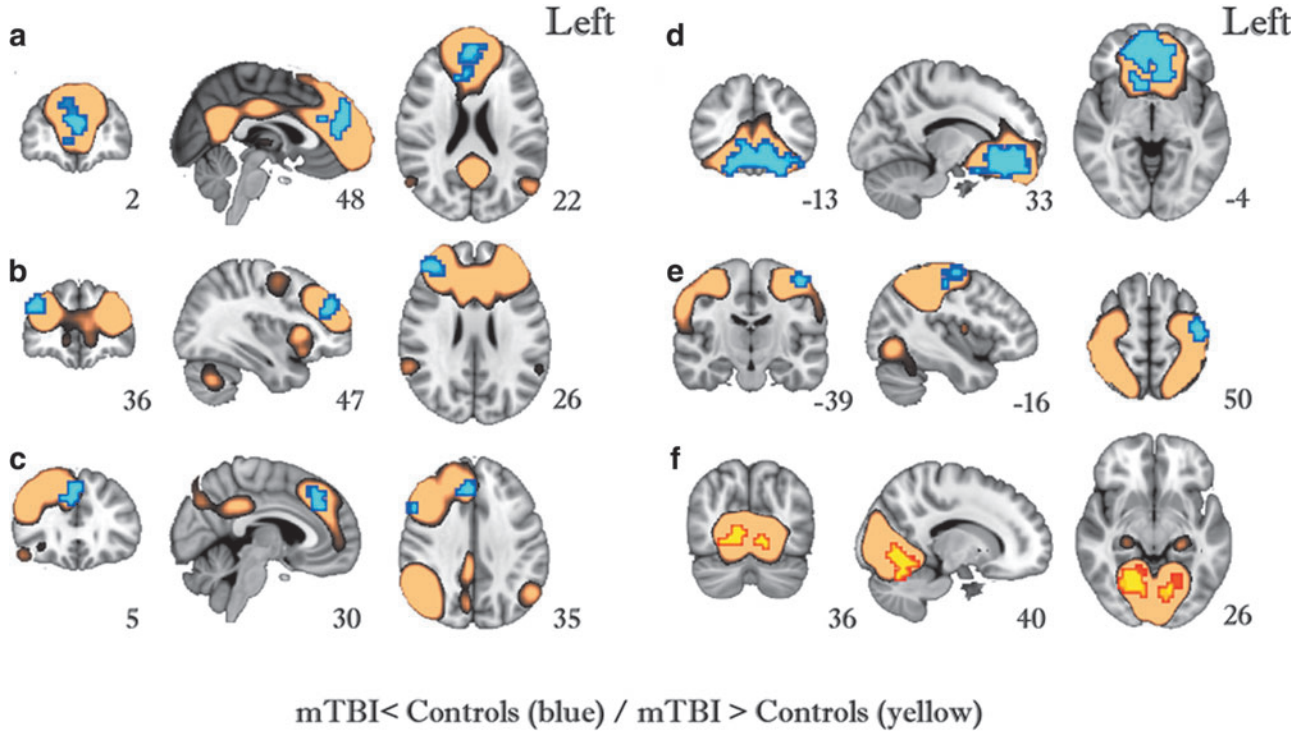

FIG. 3. Resting-state networks' (RSNs') significant differences between the whole sample of mTBI patients and healthy controls. **(a)** Default mode network (DMN); **(b)** executive control network; **(c)** frontoparietal network; **(d)** orbitofrontal network; **(e)** dorsal attentional network; **(f)** visual network. In blue: reductions in connectivity. In red-yellow: increases in connectivity. Color image is available online at www.liebertpub.com/neu

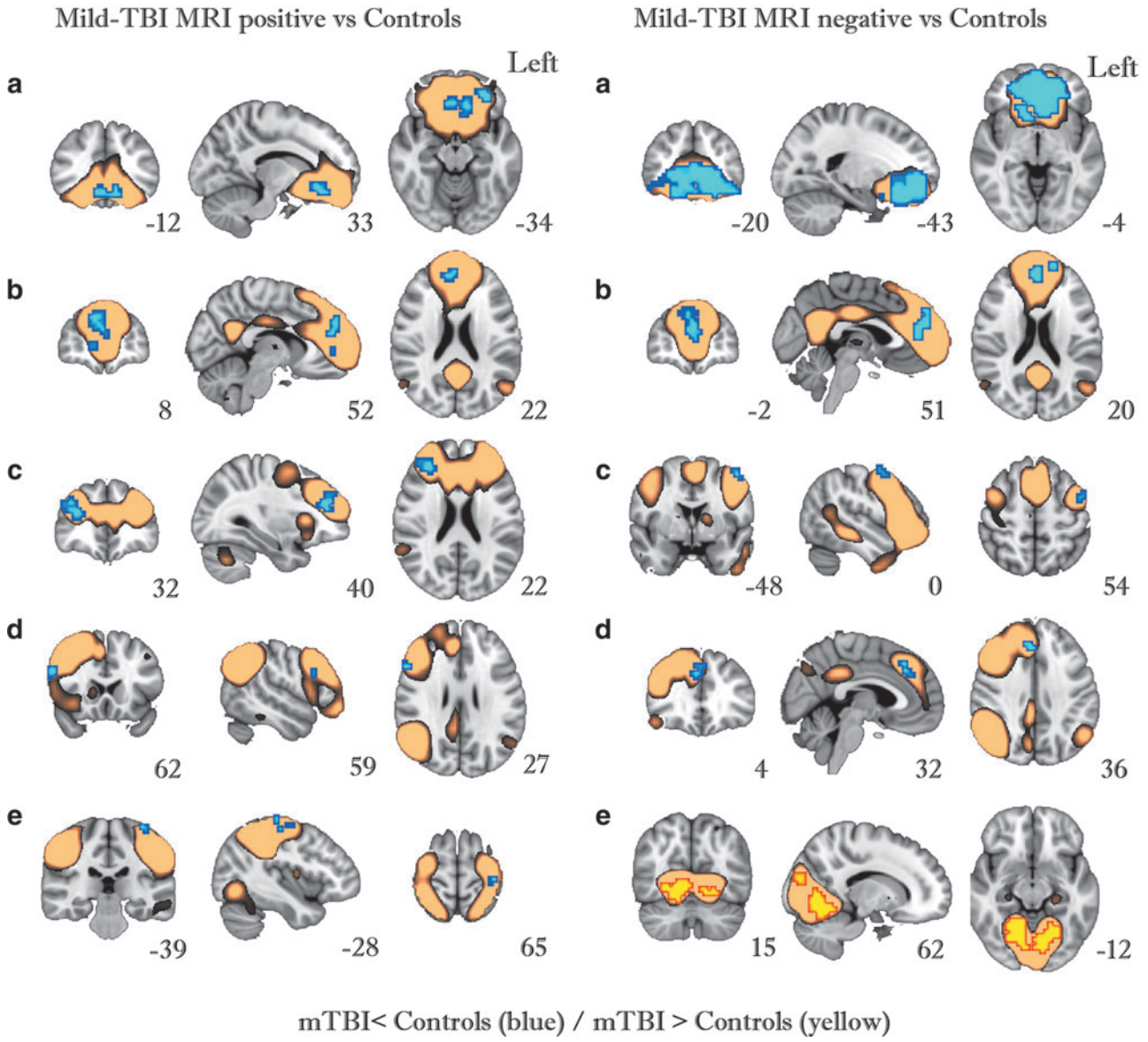

FIG. 4. Group comparisons of CT/MRI positive or negative patients versus controls. Left side: **(a)** orbitofrontal network; **(b)** default mode network (DMN); **(c)** executive control network; **(d)** frontoparietal network; **(e)** dorsal attentional network. Right side: **(a)** orbitofrontal network; **(b)** DMN; **(c)** salience network; **(d)** fronto-parietal network**; (e)** visual network. In blue: reductions in connectivity. In red-yellow: increases in connectivity. Color image is available online at www.liebertpub.com/neu

Patients with decreased connectivity presented more post-concussive symptoms.

Correlations of RSNs with 6 month cognitive performance

TMT. We found positive correlations of semiacute functional connectivity in brain regions corresponding to the DMN, salience network, and dorsal attentional network with TMT A scores at 6 months after injury in mTBI patients with CT/MRI positive scans, but not in those with CT/MRI negative scans (Fig. 6). On the other hand, in the mTBI patient group with CT/MR negative scans, we found that the measure of executive function, TMT B-A (obtained by substracting the time taken to complete the TMT-A and TMT-B), correlated positively with the connectivity corresponding to the orbitofrontal RSN.

California Verbal Learning Test. Increased semiacute connectivity within the occipito-cerebellar RSN was correlated positively with the learning memory scores at 6 months after injury in the CT/MRI negative subgroup.

All results were corrected for multiple comparisons by using FWE correction at $p < 0.05$.

Temporal interactions between RSNs

Group comparisons. After multiple comparisons correction, reduced inter-network functional connectivity in mTBI patients versus controls was found between different pairs of RSNs (Fig. 7).

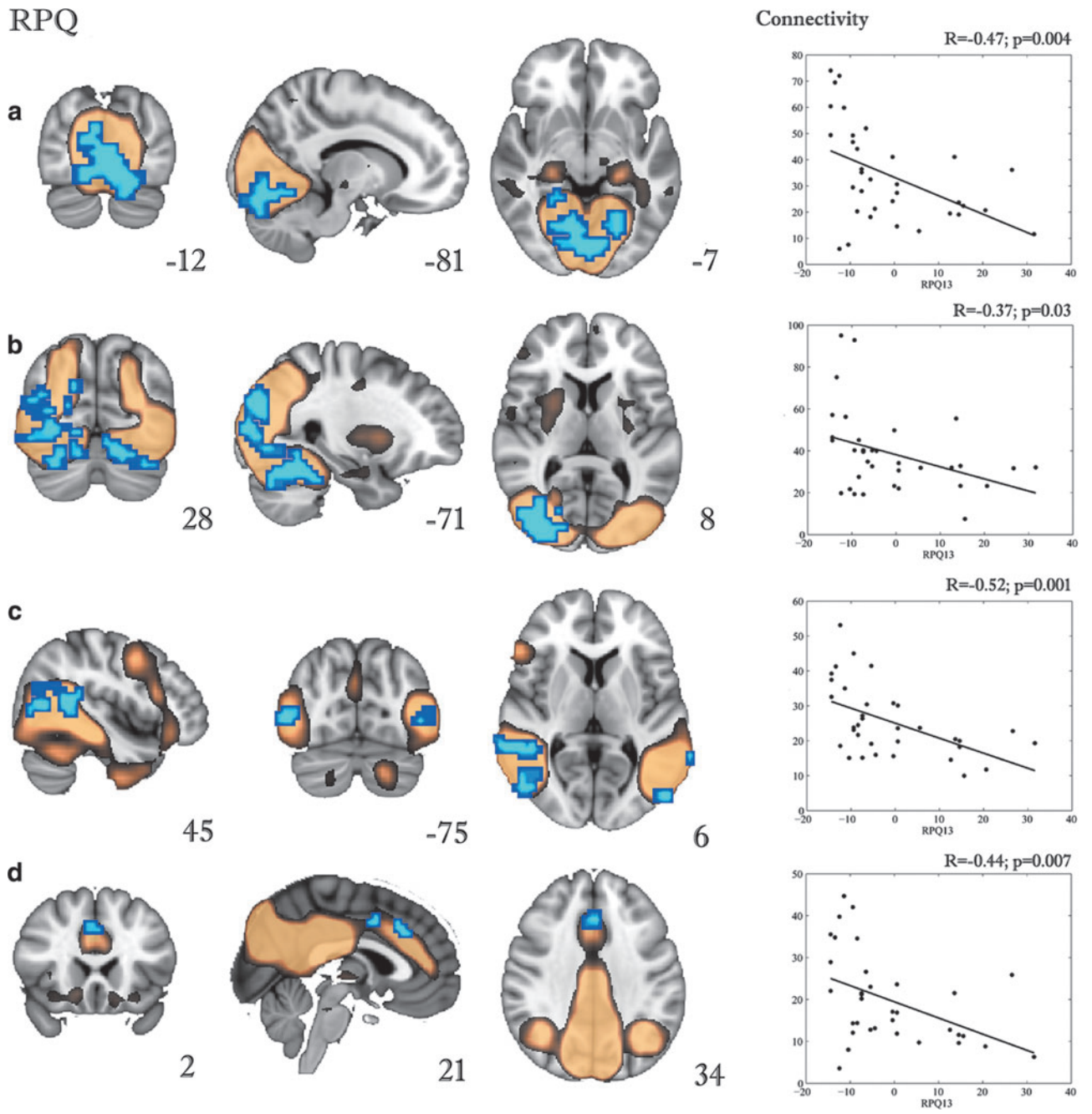

FIG. 5. Rivermead Post Concussion Questionnaire versus functional connectivity in the CT/MRI negative mTBI subgroup. **(a)** Visual network; **(b)** occipito-cerebellar network; **(c)** dorsal visual stream; **(d)** posterior default mode network. In blue: negative correlations with the behavioral test. Scatter plots show individual mean values for connectivity within the significant areas, in relation to the demeaned results of the behavioral test. Color image is available online at www.liebertpub.com/neu

Reduced network connectivity interactions were found in the CT/MRI positive subgroup of mTBI patients versus controls between two pairs of networks: 1) the auditory network with the ventral attentional network ($p = 0.04$), and 2) basal ganglia network with dorsal attentional network ($p = 0.016$).

We also found reduced network connectivity interactions in the CT/MRI negative subgroup of mTBI patients versus controls in one pair of networks: the visual network with the dorsal and ventral visual stream network ($p = 0.04$).

No differences were found when comparison was made between mTBI subgroups.

Correlations with cognitive measures. Correlations were found only in the CT/MRI negative subgroup of mTBI patients with the TMTB-A. This measure of executive function was found to correlate negatively with one pair of networks: the basal ganglia network with the orbitofrontal network ($p = 0.04$).

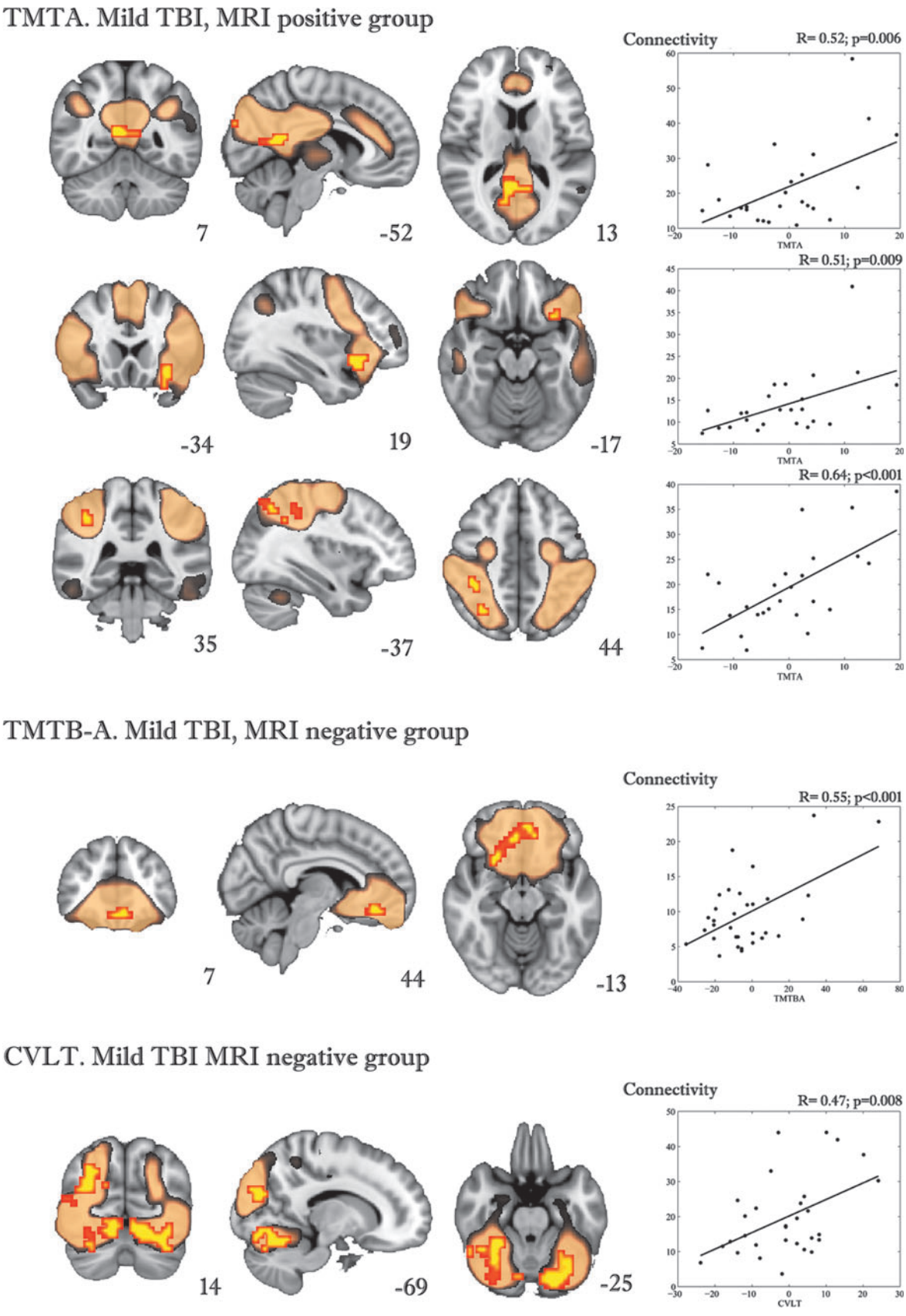

FIG. 6. Increases in connectivity related to cognitive measures in the mild traumatic brain injury (mTBI), MRI negative group. Trail Making Test A (TMT-A), CT/MRI positive mTBI: **(a)** default mode network (DMN); **(b)** salience network; **(c)** dorsal attention network. TMTB-A, CT/MRI negative mTBI: orbitofrontal network. California Verbal Learning Test–(CVLT), CT/MRI negative mTBI: occipito-cerebellar network. In red-yellow: positive correlations with the cognitive test. Scatter plots show individual mean values for connectivity within the significant areas in relation to the de-meaned results of the cognitive tests. Color image is available online at www.liebertpub.com/neu

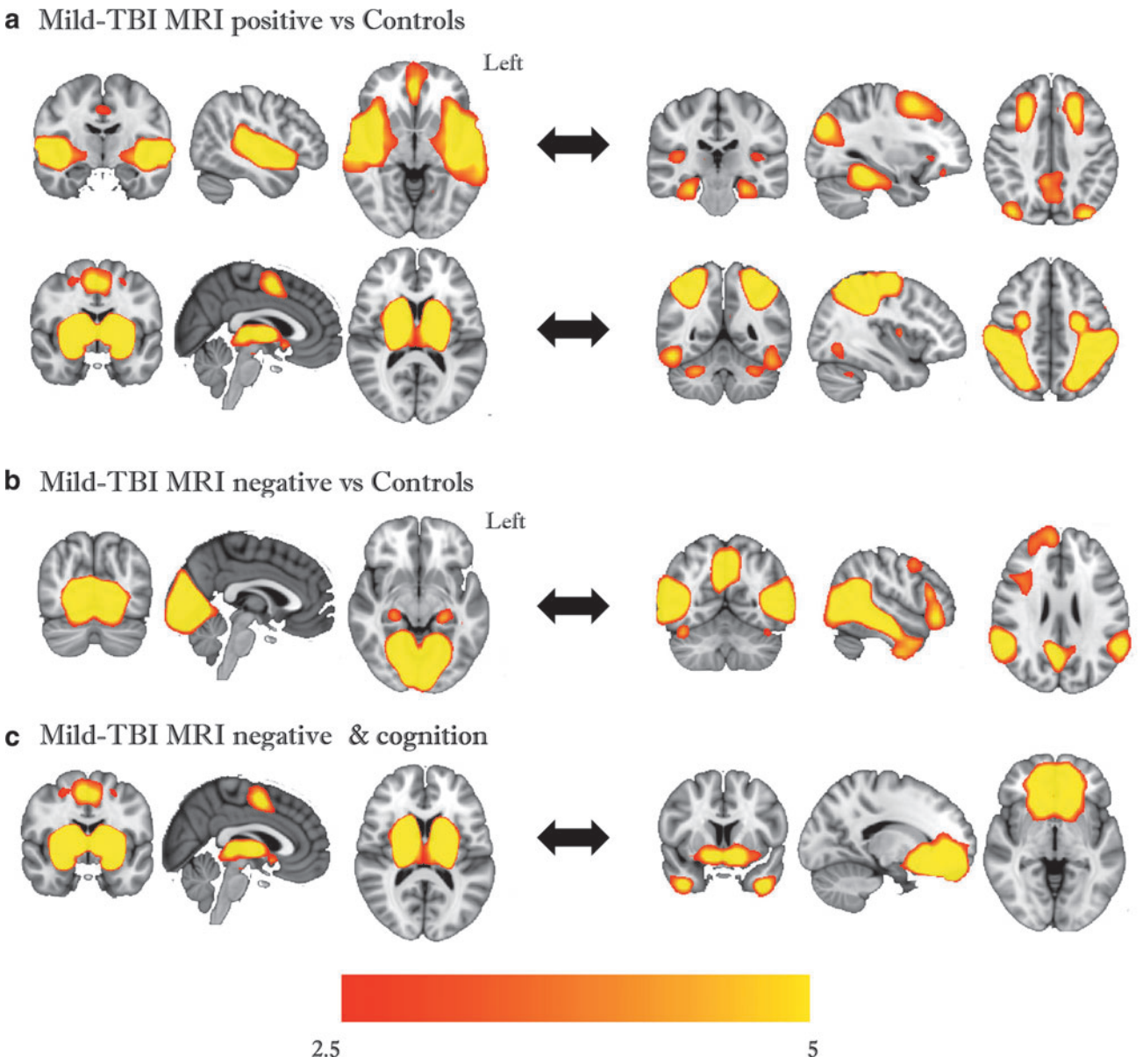

FIG. 7. Temporal interactions between resting-state networks (RSNs). **(a,b)** Pairs of networks with reduced functional connectivity in mild traumatic brain injury (mTBI) patients positive/negative versus controls. **(c)** Negative correlation between a pair of networks and the executive function measure. Color image is available online at www.liebertpub.com/neu

Discussion

To our knowledge, this is the first study to relate alterations in early resting-state functional connectivity to long-term postconcussive symptoms and cognitive outcome in a large and clinically well-defined mTBI sample. The main findings of this study are that: 1) patients with mTBI in the semiacute stage, with CT/MRI scans either positive or negative, have alterations in the connectivity of the most representative RSNs that are associated with cognitive performance at 6 months after injury; 2) patients with CT/MRI negative scans show reduced RSN connectivity that predicts postconcussive symptoms; and 3) each subgroup of mTBI patients presents a different pattern of network interaction alterations.

Previous resting-state studies have discovered both reductions and increases in connectivity in some networks after mTBI. In agreement with our study, decreases in DMN functional connectivity have been found in mTBI and postconcussive patients.[6,8] In addition, some authors have reported increases in this network in the rostral anterior cingulate and ventrolateral prefrontal cortex.[6]

Zhou and coworkers[7] showed reduced connectivity in the posterior cingulate and parietal cortex together with an increase in the connectivity in the medial prefrontal cortex. The increased connectivity of the posterior and anterior brain nodes of the DMN correlated positively with neurocognitive dysfunction. In our study, when the whole group of mTBI patients was taken together, we found reductions in connectivity mainly in the frontal areas of the DMN, the orbitofrontal network, the frontoparietal networks bilaterally, and the left parietal dorsal attentional network. Only the visual network was found to have increased connectivity. Alterations in these resting-state networks might interfere with several

cognitive functions, as they have been described as being involved in processes such as internal focus of attention, social cognition, inhibition, memory, divided attention, emotion, and language.[5]

Similarly, Stevens and coworkers[15] also assessed alteration in resting-state connectivity in mTBI patients with negative MRI scans. They studied the DMN, cognitive control networks, motor networks, and visual processing networks, finding increases and decreases in resting-state connectivity in all the studied networks. Discrepancies between our study and theirs might be explained in terms of sample sizes and the time interval between injury and performance of scanning in the mTBI patients. Our patients were scanned within 3–18 days of their injury, whereas Stevens and coworkers scanned their patients 13–136 days from injury. The longer window between injury and scan could account for the differences in the studies, as the patients were at different stages in their biological recovery from injury. Moreover, comparison of their results with ours was confounded not only by the sample differences, but also by differences in the method of analysis. Although both studies used ICA to define the networks, we restricted our analysis to within the masks of the networks, whereas Stevens and coworkers examined the connectivity differences in brain areas out of the network masks or across the whole brain.

Shumskaya and coworkers[14] studied functional connectivity at rest in the most common resting-state networks reported in the literature using ICA. The patients examined were a homogeneous sample of MRI positive mTBI patients with fronto-occipital impact injuries scanned in subacute stage. They found reductions in connectivity in the motor-striatal network and the frontoparietal network. Although their sample of patients showed deficits in some behavioral and cognitive areas compared with the control group, they did not find specific correlations with the behavioral and cognitive scores and functional connectivity within the affected RSNs.

When we compared each of the mTBI groups separately (CT/MRI positive vs. negative scans) to the healthy control group, with a few exceptions, the connectivity reductions found corresponded to the same networks that emerged from the whole-group comparison analysis. One exception is that the reductions in the orbitofrontal network for the mTBI negative MRI group were greater in extent than those for the mTBI MRI positive group. In contrast, the increased connectivity found when the visual network in the whole mTBI group was compared with the control group was solely attributed to the mTBI negative group.

With regard to the behavioral measures, we found a relationship between postconcussive symptoms at 6 months measured using the RPQ, and reductions in the connectivity of several resting-state networks in mTBI patients with MRI negative scans. The pattern of decreased connectivity that was predominantly correlated with the symptomatology was observed in posterior brain regions involving parieto-occipital areas, with the exception of a reduction in the anterior cingulate. It is particularly interesting that we found that a reduction in the visual network connectivity was correlated with behavioral symptomatology, whereas this network showed increased connectivity in the group comparisons performed between the mTBI negative group and the control group. This increased connectivity might be interpreted as compensation for injury, although we cannot tell to what extent this increase results in more efficient brain functioning, as behavioral symptoms remained present 6 months after injury. We did not find results in the network connectivity of the CT/MRI positive group of patients associated with behavioral symptoms. We suspect that this may be because of the difference in sample size we have between the mTBI MRI positive and negative groups, but it may also be a result of the heterogeneous distribution of the focal brain lesions of the mTBI CT/MRI positive group.

In addition to behavioral symptoms, we found associations among attention, executive and memory performance, and the connectivity of some networks of the two mTBI groups. In the mTBI CT/MRI positive group of patients, performance in attention and processing speed were found to be related to increases in connectivity in the DMN, the salience network, and the dorsal attentional network. Interestingly, the DMN has been shown to correlate negatively with the salience network and the dorsal attention network in healthy subjects,[24] and the DMN is associated with successful attentional response in TBI patients with different levels of severity.[25,26] Our results involving the DMN in the cognitive tasks could be also understood within the recently explored idea stating that the DMN is not only a ''task negative'' network that deactivates during goal-directed tasks, but also an active network contributing to task performance.[27–29] For example, in recent articles by Vatansever and coworkers, the authors revealed how the DMN actively interacts between various large scale connectivity networks, possibly through global integration of the information, when increasing the environmental demand of a cognitive task such as working memory.[29,30] In the mTBI CT/MRI negative group, increases in connectivity in the orbitofrontal network were related to executive function. This measure involves attention and inhibition, working memory, and mental flexibly. These cognitive skills rely on frontal circuitries, including orbitofrontal connections, especially when inhibition is involved. Increases in connectivity of the occipito-cerebellar network in this last group of patients were also found to be related to good learning performance. Overall, in the absence of control cognitive testing data, we can only presume that these increases in connectivity would favor successful performance as compensation is made for the effect of the reduced connectivity found in networks closely involved in attention and executive functions.

The interaction among resting-state networks is thought to be critical for cognition, suggesting that an imbalance between the connectivity dynamics of different networks can alter cognitive function and behavior.[31] Our study has used a novel approach to explore how brain functional resting-state networks interact. We believe that this increases understanding of how the brain produces complex behaviors. As an example, previous studies using other methodologies have found that the alteration in the network interaction between the DMN and the salience network was associated with cognitive alterations after TBI.[10,32] We found reduced interactions in both CT/MRI positive and negative mTBI groups compared with controls in several pairs of networks. For the CT/MRI mTBI positive group, dorsal and ventral attentional networks were found to have reduced connectivity with the basal ganglia and the auditory network, respectively, when compared with the control group. These alterations in the interactions between the main attentional networks could provide further information as to how patients increase connectivity in the dorsal attentional network when performing an attentional cognitive test. On the other hand, the CT/MRI mTBI negative group followed a different pattern of reduced network interactions involving the dorsal and ventral visual stream and the primary visual network. Further, in this group, the reduced interactions found between the basal-ganglia and orbitofrontal networks were associated with executive performance. The orbitofrontal, basal ganglia, and visual networks in the CT/MRI negative mTBI group of patients seemed to play an important role in behavior and cognitive performance. The orbitofrontal

cortex is the neocortical extension of the limbic system, and the medial division of the orbitofrontal circuit projects to basal ganglia structures and, therefore, is involved in the determination of the appropriate environmentally elicited behavioral responses. Lesions in this area can result in behavioral disinhibition and emotional lability.[33] Moreover, the connectivity of the basal ganglia with the frontal cortex and with posterior visual areas have been reported to have a role in successful attention shifting (van Schouwenburg).[34]

It is of note that in our study we used nonparametric statistics based on permutation testing with a threshold-free cluster enhancement (TFCE) method to correct for multiple comparisons. In the TFCE method, combined with permutation testing using the FSL randomize function, the cluster-level threshold is not defined ''*a priori*'' as criticized in the recent fMRI literature.[35] This TFCE method combined with nonparametric permutation testing is accepted as the recommended approach for cluster-level inference for neuroimaging studies.[36]

Conclusion

In conclusion, despite the questions that remain to be clarified, the overall findings of our study show widespread alterations of functional connectivity within and between resting- state networks in the semiacute phase after mTBI, with significant relationships to long-term symptoms as well as to behavioral and cognitive outcomes. We further demonstrate some conserved and some different patterns of altered functional connectivity in those mTBI patients with focal lesions on CT and/or MRI versus those without such visible lesions. These results support the use of functional connectivity from rsfMRI as an early biomarker for mTBI diagnosis and outcome prediction, specifically for the development of persistent postconcussive syndrome.

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Author Disclosure Statement

No competing financial interests exist

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SAGE

Collaborative targeted maximum likelihood estimation for variable importance measure: Illustration for functional outcome prediction in mild traumatic brain injuries

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Abstract

Standard statistical practice used for determining the relative importance of competing causes of disease typically relies on ad hoc methods, often byproducts of machine learning procedures (stepwise regression, random forest, etc.). Causal inference framework and data-adaptive methods may help to tailor parameters to match the clinical question and free one from arbitrary modeling assumptions. Our focus is on implementations of such semiparametric methods for a variable importance measure (VIM). We propose a fully automated procedure for VIM based on collaborative targeted maximum likelihood estimation (cTMLE), a method that optimizes the estimate of an association in the presence of potentially numerous competing causes. We applied the approach to data collected from traumatic brain injury patients, specifically a prospective, observational study including three US Level-1 trauma centers. The primary outcome was a disability score (Glasgow Outcome Scale - Extended (GOSE)) collected three months post-injury. We identified clinically important predictors among a set of risk factors using a variable importance analysis based on targeted maximum likelihood estimators (TMLE) and on cTMLE. Via a parametric bootstrap, we demonstrate that the latter procedure has the potential for robust automated estimation of variable importance measures based upon machine-learning algorithms. The cTMLE estimator was associated with substantially less positivity bias as compared to TMLE and larger

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coverage of the 95% CI. This study confirms the power of an automated cTMLE procedure that can target model selection via machine learning to estimate VIMs in complicated, high-dimensional data.

Keywords
Variable importance measure, causal inference, high-dimensional data, semi-parametric, collaborative targeted maximum likelihood, positivity

1 Introduction

Variable importance measures (VIM) are used to rank the importance of each of a set of explanatory variables (e.g. competing causes) in predicting an outcome. Standard estimation methods rely on ad hoc techniques such as multivariate regressions and associated stepwise procedures,[1] penalized regression (e.g. lasso[2]), recursive partitioning methods[3] or random forest.[4] Most of these methods are constrained by assuming parametric models, while others such as the random forest produce VIM that are not typically robust nor directly interpretable by clinicians. Moreover, none of these methods is ''targeted'' to estimate specifically variable importance, but such importance measures are simply byproducts of a prediction algorithm. The approach we advocate defines VIM by analogous causal parameters, such as the average treatment effect (ATE), and then estimates this parameter for each variable separately.[5] Under a set of causal assumptions, this approach has the virtue of estimating causal variable importance rather than associations. In addition, it offers the possibility to use data-adaptive machine learning methods for the relevant prediction models.

Targeted maximum likelihood estimation (TMLE) is a general multistep procedure to produce substitution estimators with robust inference and optimal asymptotic properties.[5] TMLE estimates for variable importance analysis tend to be stable at large sample sizes.[6] However, if there are practical positivity violations (that is, for some sets of individuals defined by values of covariates, there is little to no experimentation in the variable of interest[7]), TMLE performance can be dismal. We hypothesize that collaborative targeted maximum likelihood estimation (cTMLE), which is an important modification of the TMLE procedure particularly well-suited to finite samples, might help to overcome this problem and thus provide a true automated machine for variable importance.[8–10] cTMLE, for each variable of interest (one at a time), automatically selects the set of adjustment variables using appropriate dimension reductions, so that the bias-variance trade-off for estimating variable importance is optimal.[11] The purpose of our study was to evaluate the performance of cTMLE for analyzing high-dimensional data and return a ranked list of variable importance estimates with associated robust inference. For this purpose, we analyzed a prospectively collected dataset of patients suffering from traumatic brain injury (TBI),[12] with the goal of finding the most important variables among pre-injury, injury-related factors, routine clinical, biological and radiological factors, to predict global functional recovery, as measured by the Glasgow Outcome Scale - Extended (GOSE) disability instrument,[13] following mild TBI.

2 Variable importance measure

The goal of the methodology is to define variable importance as a parameter that can be estimated data-adaptively, but maintains both relevant clinical interpretations, and also desirable asymptotic statistical properties. Thus, we start with discussing formalities regarding the data-generating model and definitions of our proposed VIM. Then, we discuss straightforward substitution methods,

as well as important modifications of these (TMLE and cTMLE) resulting in a fully automated, data-adaptive (machine learning) procedure.

2.1 Defining a variable importance measure as a substitution estimator

Consider our data to be (Y, X), where Y is the outcome and X is a vector of predictors, $X=X_1,\ldots,X_p$. Start by defining $X_j^* = I(X_j \in S_j)$ and $X_{-j} = (X_1, X_2, \ldots, X_{j-1}, X_{j+1}, \ldots, X_p)$ that is X_j^* is the indicator that the variable X_j in some subset S_j (for which we are willing to estimate the VIM) and X_{-j} simply includes all the remaining covariates in their original form. Note that in the case of a model that defines a time-ordering ($X_{-j} \rightarrow X_j^* \rightarrow Y$), then the VIM based on adjustment association of X_j^* and Y has particular causal interpretation.[14]

We define our VIM parameter to be

$$VIM_j = E_{X-j}\left\{E\left(Y|X_j^* = 1, X_{-j}\right) - E\left(Y|X_j^* = 0, X_{-j}\right)\right\} \quad (1)$$

Note that if one assumes the above time ordering, along with other assumptions (e.g. positivity), equation (1) identifies the causal parameter $E(Y(1)\text{-}Y(0))$, where $Y(a)$ is the so-called counterfactual outcome had everyone in the population been set to $X_j^* = a$. Note that if some of the X_{-j}'s precede X_j^*, whereas others are after, then equation (1) might still identify a causal parameter, but a more complicated one. Given we are not asserting a time ordering among the X's, we do not emphasize the correspondence with causal effect estimation, but the VIM above is still an interesting variable importance parameter, which can be identified in a nonparametric model, that is, without constraints on how we estimate the regression of Y versus X.

If $\hat{Q}_j(X_j^*, X_{-j}) = \hat{E}(Y|X_j^*, X_{-j}.)$ is the estimated regression of Y versus the covariates, then a simple substitution estimator is defined as

$$\hat{VIM}_j = \frac{1}{n}\sum_{i=1}^{n} \hat{Q}_j(1, X_{-j,i}) - \hat{Q}_j(0, X_{-j,i}) \quad (2)$$

with subscript i referring to the observations and j refers to the predictor.

To make the analogy to standard methods, such as simple linear regression of Y versus X, note that if $Q_j(X_j^*, X_{-j}) = \beta_0 + \alpha.X_j^* + \beta_1.X_1 + \cdots$, then $VIM_j = \alpha$. Our goal is to avoid such biased parametric assumptions, use procedures much more powerful for fitting the data, but still result in a relatively interpretable VIM for which the estimation can be automated. In the next section, we discuss an optimal data-adaptive method for fitting the regression model Q_j.

2.2 Super learner

The algorithm used to estimate the regression plugged into equation (2) should be estimated based on some principles of optimality, and should be flexible enough to allow for very simple or very complex models. The *Super Learner* (SL) is such an algorithm, which has been proposed as a method to select an optimal regression algorithm from among a set of candidates (or library) using cross-validation.[15–17] We used the SL (available in R[18]) with 10 splits for the V-fold cross-validation step and the following parametric and non-parametric algorithms were included in the Super Learner library: logistic regression both including only main terms for each covariate and also including interaction terms[19]; Stepwise logistic regression using a variable selection procedure based on the Akaike Information Criterion[20]; Bayesian generalized linear model[21], Random Forest[4] and Neural

Networks.[22] The risks associated with the different estimators were evaluated using the cross-validated mean squared error (MSE).

The next step is to determine the importance of each variable to predicting the outcome. This is a particular challenge because although the SL has optimality properties with regards to prediction, it offers no direct way of interpreting the model to determine which variables are most important in prediction. Substitution estimators[23–25] are based on using such "black box" algorithms to predict the distribution of the outcome at different levels of the current variable of interest, while keeping the others fixed at their observed values. Specifically, the SL can be used to estimate $E(Y|X_j^* = 1, X_{-j})$ and $E(Y|X_j^* = 0, X_{-j})$ for each individual. Then, the difference in the mean predicted outcome at different levels of the prediction variable could be used as a summary of its independent association with the outcome as in equation (2). However, even if SL is optimal at estimating $E(Y|X_j^* = 1, X_{-j})$ and $E(Y|X_j^* = 0, X_{-j})$, there is no guarantee that it will be optimal at estimating the difference between the two, which is precisely our quantity of interest. This needs an additional targeting step, which may be realized by using targeted maximum likelihood estimators as detailed in the next section.

2.3 TMLE and cTMLE

The TMLE approach involves fluctuating the initial estimate of Q_j into an updated estimate Q_j^*, in order to make a bias/variance tradeoff targeted towards the parameter of interest. TMLE is consistent and asymptotically normally distributed under regularity conditions, when either one of these two factors of the likelihood is correctly specified, and it is efficient if both are correctly specified.[8,9]

Practically, TMLE in this context is a two-step procedure: *first* running an initial regression to fit $Q_j(X_j^*, X_{-j})$ i.e. the expected value of the outcome given the covariate of interest (i.e. the candidate risk factor in our situation) and adjusting for all other covariates. We emphasize Super Learning for this step. The next step is an update of the initial regression by (a) getting the predicted value from the initial estimator, which will be used as an offset, (b) deriving a so-called clever covariate $h_j(X_j^*, X_{-j}) = \left[\frac{I(X_j^*=1)}{g_j(X_{-j})} - \frac{I(X_j^*=0)}{1-g_j(X_{-j})}\right]$ where $g(X_{-j}) \equiv P(X_j^* = 1|X_{-j})$ and (c) regressing the outcome against this covariate and the offset: $\hat{Q}_j^*(X_j^*, X_{-j}) = \hat{Q}(X_j^*, X_{-j}) + \hat{\varepsilon}h(X_j^*, X_{-j})$ or $logit[\hat{Q}_j^*(X_j^*, X_{-j})] = logit[\hat{Q}(X_j^*, X_{-j})] + \hat{\varepsilon}h(X_j^*, X_{-j})$ if a logistic model is fit, e.g. for a binary Y. Note that one must fit a model for $g(X_{-j})$ as well, and this can also involve Super Learning; we discuss how to automate variable selection in this model below as it can have serious consequences for efficiency in finite samples. The TMLE estimator of the parameter of interest is then estimated, just as equation (2), but with the updated regression of Y versus the covariates

$$\hat{VIM}_j^{TMLE} = \frac{1}{n}\sum_{i=1}^{n} \hat{Q}_j^*(1, X_{-j,i}) - \hat{Q}_j^*(0, X_{-j,i}) \tag{3}$$

In the situation of variable importance measure, this parameter must be estimated for each variable in the subset X_j^* of X. Hence, a separate procedure/estimate is conducted for each X_j^*, considering this X_j^* as the exposure and the others X_{-j} as covariates. Standard errors are calculated using the influence curve, and the central limit theorem[5] can be applied to derive the typical measures of uncertainty (p-values, confidence intervals, etc.).

2.4 Finite sample performance and practical positivity

If all datasets had ''sufficient'' sample sizes, then this TMLE for VIM results in a potentially automated procedure. However, for such ambitious parameters (particularly when there are many covariates), the machine learning fits of the required regressions can result in TMLE estimators that behave poorly in finite samples (significant bias, non-normal sampling distributions, etc). One of the most important causes of non-robust performance is practical violation of the positivity assumption.[7] The positivity assumption requires that all possible combinations of covariates X_{-j} must be observable for all levels of X_j^*; concisely, it is required that $0 < g_j(X_{-j}) \equiv P(X_j^* = 1 \big| X_{-j}) < 1$ over the distribution of X_{-j}. In the situation of finite samples, and especially when the set of X_j^* is large, there is likely to be some ''practical'' positivity assumption, meaning that in the sample, there are groups of subjects defined by close values of X_{-j}, that have little to no experimentation in the covariate of interest, X_j^*. Given that we are estimating the VIM separately for each variable of interest, it is likely that, for some of them, the practical positivity assumption will not hold. In this case, the updating step of the TMLE procedure, which relies on estimating g_{j} to calculate the clever covariate, may fail to produce consistent, robust estimators. Note that this would be true for alternative estimators based on g_{j}, i.e. inverse probability of treatment weighted (IPTW) and the doubly robust versions (DR-IPTW[26,27]). Thus, one would need a procedure that automatically adjusts the ''complexity'' of the estimator to optimize the variance-bias trade-off, based upon the information in the data for the parameter of interest.

The best bias/variance tradeoff for g_j might not coincide with the optimal final estimate especially when X is of high dimension. Thus, there is a need for an automated procedure that would tailor the fit of g_j to optimize the final estimate. Such a procedure has only recently been proposed and is called collaborative targeted maximum likelihood estimation (cTMLE).[10]

The cTMLE is an extension of the TMLE methodology.[10,11] It also involves a *targeted* model selection step for the nuisance parameter portion of the likelihood g_j in order to get the most efficient estimate of variable importance. A cTMLE estimator is constructed by building a family of candidate estimators, then choosing the ''best'' among them, using the cross-validated risk of the resulting augmented model $\hat{Q}_j^*$. The template for construction of the cTMLE estimator is described in detail in Chapter 19 of Van der Laan and Rose.[5] This procedure creates an entire sequence of candidate TMLE based on an initial estimate for $Q_j(X_j^*, X_{-j})$ coupled with a succession of increasingly non-parametric estimates for $g(X_{-j}) \equiv P(X_j^* \big| X_{-j})$. The evolution with TMLE where g_j may be estimated data-adaptively is that cTMLE estimates of g_j are constructed based on a loss function for the TMLE of the relevant factor Q_j that uses the nuisance parameter to carry out the fluctuation, instead of a loss function for the nuisance parameter itself. Likelihood-based cross-validation is used to select the best estimator among all candidate TMLE estimators of Q_j in this sequence. Theoretical results have demonstrated that the cTMLE is consistent and asymptotically normally distributed even when Q_j and g_j are both misspecified, provided that g_j solves a specified score equation implied by the difference between the Q_j and the true Q_j.[10,11]

In summary, combining an initial SL fit with cTMLE results in a ''machine'' that may be used for robust estimation of our VIM parameter. Similarly to TMLE, inference is also based on the influence curve and the confidence intervals are constructed by applying the central limit theorem. In this case, one not only does not need re-sampling based inference procedures such as the bootstrap (which can dampen the performance of such an intensive data-adaptive procedure), but in fact conditions for the consistency of bootstrap are much more restrictive and often fails in practice. Thus, deriving inference, given it only relies on calculation of the influence curve once after estimation is completed, is essentially computationally free.

In our example, the statistical analysis consisted of examining the relative influence of each explanatory variable on the GOSE at three months (i.e. variable importance measure). For both Q and g_j, we used Super Learner.[15] Such predictions were used to derive the TMLE and the cTMLE estimators for variable importance measure. All analyses were run on R 2.15.2 statistical software running on a Mac OsX platform (The R Foundation for Statistical Computing, Vienna, Austria), using the packages Super Learner,[18] cvAUC,[28] and TMLE.[29]

2.5 Estimator performance

We defined the bias in the VIM estimator as $Bias(VIM_j) = E(VIM_j) - VIM_{0,j}$ where $VIM_{0,j}$ is the true value of the particular variable importance measure. Bias in an estimator can arise due to a range of causes. Among them, we focused on the bias related to the positivity assumption violation (positivity bias). Our objective was to quantify the positivity bias associated with each estimator (TMLE and cTMLE).

The variables for which the TMLE was potentially biased were identified by comparing the TMLE and cTMLE estimates, any important discrepancy between the two estimates indicating that one of the two estimators has to be biased. For those variables with strong differences, we quantified the extend to which such difference could be explained by the positivity bias. To quantify the bias related to positivity violation, we used a simulation procedure proposed by Petersen et al.[7] that relies on parametric bootstrap. This procedure does not rely on some arbitrary data-generating distribution but instead aims at recreating samples from a distribution as close as possible to the actual data-generating distribution. The bias as quantified by the parametric bootstrap can be written as $Bias(VIM_j) = E_{Pboot}(VIM_{b,j}) - VIM_j$ where $\hat{P}_{boot}$ is the bootstrap distribution (defined by repeated draws from empirical distribution), and $VIM_{b,j}$ are the estimates from each of the $b = 1,\ldots,B$ bootstrap samples. The candidate estimators (in our case, the TMLE and the cTMLE estimators) are applied to each bootstrapped dataset. In the bootstrap samples, the bias is defined as the difference between the mean of the resulting estimates across datasets and the true parameter value for the bootstrap data generating distribution. Specifically, one first estimates both Q and g from the data as discussed above. Then, using these estimates, one generates new random data sets in same way the original data set was assembled (e.g. random draws). By using the same estimation procedure as in the original sample, one can examine the sampling distribution of competing estimators in a world where one treats the estimated Q and g as the true distributions. Given that one estimates the models in these random parametric bootstrap draws assuming the same algorithms used to construct the ''true'' distribution, the estimators are guaranteed to be consistent unless g fails to satisfy the positivity assumption. As a result, the parametric bootstrap provides an optimistic estimate of finite sample bias, in which bias due to model misspecification other than truncation is eliminated.

Bootstrap-based simulations were also used to compute the coverage of the 95% confidence intervals (95% CI) for each estimator, defined as the proportion of time the confidence interval contained the true value set by simulation. In addition, in order to quantify to extend to which the coverage was affected by the bias in the point estimates rather than the variance estimator performance, we computed the bias-adjusted coverage of the 95% CI as the area under N(0,1) density between $-1.96 - \hat{b}_n$ and $1.96 - \hat{b}_n$, with b_n being the normalized bias defined as $b_n = \frac{Bias(VIM_j)}{SE(VIM_j)}$, that is the estimated bias divided by the standard error.

In order to explore whether the benefit associated with cTMLE in case of positivity violation was dependent of the sample size, we reran the parametric bootstrap to simulated new datasets with decreasing sample size ($n = 1000$; 500; 100; 50). The positivity bias was recalculated for each

sample size. Because such simulations are computationally intensive, they were only performed for a single variable, the one associated with the largest positivity bias.

3 Application on TRACK-TBI data

3.1 TRACK-TBI

The Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) study aimed to prospectively examine the influence of pre-injury factors, injury-related factors, and some routine clinical, biological and radiological factors on the functional outcome following TBI, as evaluated by the GOSE at three months.[13] This study is a prospective, multicenter, observational cohort study including patients referred to the emergency department (ED) of three Level-I trauma centers in the USA (San Francisco General Hospital, University of Pittsburgh, and University Medical Center Brackenridge) for TBI. Institutional review boards of the three participating centers approved all study protocols, and all patients or their legal representatives gave written informed consent. Inclusion criteria for TRACK-TBI Pilot were acute external force trauma to the head, presentation to the ED within 24 h of injury, and sufficient indications for a clinical head CT to assess for traumatic intracranial injury using the American College of Emergency Physicians/Centers for Disease Control (ACEP/CDC) evidence-based joint practice guideline.[30] Exclusion criteria were pregnancy, comorbid life-threatening disease, incarceration, or serious psychiatric and neurologic disorders that would interfere with outcome assessment. Non-English speakers were not enrolled due to inability to participate in outcome assessments, which are normed and administered in English. As our analysis is focused on mild TBI, we included only patients with complete three-month GOSE, and mild TBI as defined by the clinical standard of ED admission GCS of 13 to 15. For clinical relevance, three different populations had to be considered separately: (1) the overall population ($n = 365$); (2) the population of patients with genetic information ($n = 261$); and (3) the population of patients without any PTSD six months after injury ($n = 188$). Thus, though a large study of its kind, the questions of interest, and numbers of variables involved, were relatively large for such modest sample sizes. Therefore, relying on data-adaptive procedures might be misleading, inspiring procedures that could adjust the estimation/inference for the available sample size.

3.2 Outcome measures and covariates

The primary outcome measure for this study was the eight-point GOSE at three months post-injury, obtained through structured interview with each participant by research assistants trained to uniformly assess the GOSE. The GOSE is a well-validated, widely employed summary assessment of global function after TBI suitable for clinical trials.[13] Secondary outcome measures included GOSE at six months, mortality at hospital discharge and three-month outcome, as well as presence of post-traumatic stress disorder (PTSD) symptoms measured by the PTSD Checklist (civilian version, PCL-C) and classified by the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) PTSD criteria at six months.[31]

The goal was to estimate VIM with potential relevant clinical interpretations for many potential competing causes of the outcome which can be categorized as (1) pre-injury factors (e.g. age, gender, medical history, prior anticoagulant drugs, psychiatric history, previous TBI, educational level, marital status, and employment status), (2) injury-related factors (e.g. trauma mechanism, PTA, loss of consciousness), (3) clinical factors (e.g. GCS, injury severity score,[32] heart rate, blood pressure, oxygen saturation, and temperature), (4) biological factors (e.g. hemoglobin, platelet

count, and blood glucose); and (5) radiological factors (Marshall score[33] and Rotterdam score[34]). Information concerning some genotypic single nucleotide polymorphisms such as the ankyrin repeat and kinase domain containing 1 (ANKK1) candidate gene (rs1800497) involved in dopamine transmission of the dopamine D2 receptor and the Apolipoprotein E (ApoE) gene (rs7412, rs429358) was available for 270 patients. The variables explored as potential risk factors had to be dichotomized as follows: Marshall grade = 1 vs. >1; Rotterdam grade ≤2 vs. >2; GCS = 15 vs. <15; systolic blood pressure <90 mmHg vs. ≥90 mmHg; heart rate <100 bpm vs. ≥100 bpm; respiratory rate >18 cpm vs. ≤18 cpm; oxygen saturation <94% vs. ≥94%; ApoE polymorphism E2/E4, E3/E4, E4/E4 vs. others; and ANKK1 polymorphism T/T vs. others.

3.3 Results

A total of 485 mild TBI patients have been included in the TRACK-TBI Pilot study, of whom 125 patients with missing three-month GOSE were excluded from the analysis. The remaining 365 patients were included in the analysis (Figure 1): 107(40.1%) were females and the median age was 44.[27–59] Clinical, demographic, and socioeconomic characteristics of study participants are described in the Table 1. Most patients (363, 99%) were alive at hospital discharge (Table 1). All these 363 patients were alive at three months post-injury and their median GOSE was 7.[6–8]

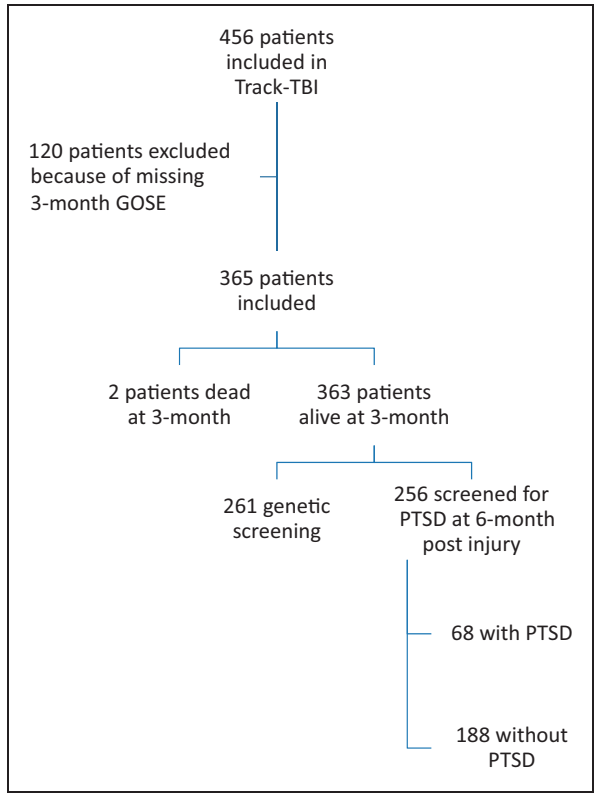

Figure 1. Flowchart.

Table 1. Clinical, demographic, and socioeconomic characteristics of the 365 study participants.

Characteristic	Value
Age (years)	44 (27–59)
Gender (female)	107 (40%)
Race	
Caucasian	302 (83%)
African-American Black	32 (8%)
Asian	18 (5%)
Other	11 (3%)
Unknown	2 (1%)
LOC	245 (67%)
PTA	226 (65%)
Employment status at time of TBI (active)	200 (55%)
Marital Status at time of TBI (married/living together)	125 (34%)
Prior TBI	186 (51%)
History of psychiatric disease	115 (31%)
Prior anticoagulant use	54 (15%)
GCS	15 (14–15)
ISS	9 (0–17)
Heart rate (bpm)	86 (76–100)
Systolic blood pressure (mmHg)	138 (127–155)
Respiratory rate (cpm)	18 (16–19)
Oxygen saturation (%)	99 (97–100)
Body temperature (°C)	36.6 (36.1–36.8)
Hemoglobin (g/dl)	14 (13–14.9)
Platelet count ($\times 1000/mm^3$)	242 (193–301)
Prothrombine time (s)	13.6 (12.9–14.2)
Blood glucose (g/L)	1.1 (1.0–1.3)
CT-Marshall category	213/132/8/4/6/2
CT-Rotterdam category	5/271/76/8/3/2
GOSE at three months post-injury	6/7/9/42/63/117/121
GOSE at six months post-injury	8/4/4/37/53/83/107
PTSD at six months post injury	68 (19%)
Three-month survival	363 (99%)

LOC: loss of consciousness; PTA: post-traumatic amnesia; GCS: Glasgow Coma Scale; ISS: injury severity score.

The presence of PTSD was assessed in 256 patients. Sixty-eight (27% of the 256; 19% of the overall cohort) had symptoms of PTSD at six months after injury. The three-month GOSE in patients diagnosed with a PTSD was lower than the GOSE in patients without PTSD [6 (5; 7) vs. 7(7; 8), $p < 0.001$].

3.4 cTMLE-based variable importance measure

Based on cTMLE, two characteristics concerning patients' medical history were associated with a poor functional outcome (Table 2): history of hepatic disease [VIM: −0.176 (−0.215; −0.136),

Table 2. Variable importance measure (VIM; equation (1)) results based on cTMLE.

Characteristic	VIM (95% CI)
History of hepatic disease	−0.176 (−0.215; −0.136)[a]
History of psychiatric disease	−0.103 (−0.156; −0.050)[a]
Prior TBI	−0.037 (−0.078; 0.003)
Prior treatment with anticoagulants	0.024 (−0.024; 0.072)
Employment status at time of TBI (inactive vs. active)	−0.066 (−0.113; −0.019)[a]
Marital Status at time of TBI (Married/living together vs. alone)	0.040 (−0.005; 0.084)
Hypotension (SBP < 90 mmHg)	−0.062 (−0.131; 0.070)
Tachycardia (HR > 100 bpm)	−0.045 (−0.083; −0.006)[a]
Tachypnea (RR > 18 cpm)	−0.020 (−0.069; 0.028)
Hypoxia (SpO_2 < 94%)	0.010 (−0.018; 0.037)
GCS (<15 vs. 15)	−0.017 (−0.065; 0.031)
Positive drug screening	−0.009 (−0.085; 0.067)
Rotterdam classification (>2 vs. ≤2)	−0.081 (−0.127; −0.036)[a]
Marshall classification (>1 vs. 1)	−0.107 (−0.153; −0.062)[a]
ANKK1 polymorphism: T/T vs. others	−0.467 (−0.879; −0.056)[a]
ApoE polymorphism: E2/E4, E3/E4, E4/E4 vs. others	0.001 (−0.307; 0.308)

Note: The estimates are adjusted for all measures confoundings and obtained using collaborative targeted maximum likelihood estimation; 95% CI: 95% confidence intervals; Apo E: Apolipoprotein E; ENT: ear, nose, throat.
[a]Statistical significance ($p<0.05$).

$p < 0.001$] and history of psychiatric disease [VIM = −0.103 (−0.156; −0.050), $p < 0.001$]. Unemployment at the time of trauma was also associated with a lower value of the three-month post-injury GOSE [VIM = −0.066 (−0.113; −0.019), $p = 0.010$]. Being married or living together at the time of injury associated with a better outcome [VIM = 0.040 (−0.005; 0.084), $p = 0.080$]. At hospital admission, a tachycardia as defined by a heart rate >100 bpm [VIM = −0.045 (−0.083; −0.006), $p < 0.001$] was associated with the three-month post-injury GOSE. CT scan abnormalities, as evaluated by the Marshall [VIM = −0.107 (−0.153; −0.062), $p < 0.001$] and the Rotterdam [VIM = −0.081 (−0.127; −0.036), $p < 0.001$] classifications, were found to be significantly associated with the three-month post-injury GOSE.

The same analysis was performed in the subsample of patients without PTSD at six months post-injury. This led to similar results for the Marshall grade [VIM = −0.205 (−0.275; −0.135), $p < 0.001$], the Rotterdam grade [VIM = −0.150 (−0.198; −0.103), $p < 0.001$] and the history of hepatic disease [VIM = −0.107 (−0.162; −0.052), $p < 0.001$], which remained the most important predictors for the three-month post-injury GOSE. However, when only considering the patients without PTSD at six months post-injury, the impact of marriage status [VIM = 0.017 (−0.038; 0.071), $p = 0.545$], employment status [VIM = −0.063 (−0.130; 0.003), $p = 0.063$] as well as psychiatric history [VIM = −0.045 (−0.114; 0.024), $p = 0.200$) on the three-month GOSE were no longer significant.

Eventually, of the 270 patients with genetic information, the ANKK1 polymorphism T/T was found to be negatively associated with the three-month post-injury GOSE [VIM = −0.467 (−0.879; −0.056), $p = 0.026$]. No significant association was found between the polymorphism of the ApoE gene and the neurological outcome [VIM = 0.001 (−0.307; 0.308), $p = 0.997$) (Table 2).

Table 3. Variables with high suspicion of positivity violation.

Characteristic	VIM(TMLE)	VIM(cTMLE)
History of musculoskeletal disease	−0.156 (−0.194; −0.118)	−0.031 (−0.078; 0.016)
History of pulmonary disease	0.009 (−0.021; 0.039)	0.045 (−0.008; 0.097)
Hypotension (SBP < 90 mmHg)	−0.323 (−0.372; −0.272)	−0.062 (−0.131; 0.070)
Gender	0.123 (0.086; 0.159)	0.035 (−0.014; 0.084)

Table 4. Experimental treatment assignment-related bias and 95% CI coverage associated with TMLE and cTMLE estimators.

	TMLE		cTMLE	
Characteristic	Positivity bias	Coverage	Positivity bias	Coverage
History of musculoskeletal disease	0.020	0.262	0.005	0.782
History of pulmonary disease	0.369	0.055	0.001	0.766
Hypotension (SBP < 90 mmHg)	0.026	0.609	0.005	0.833
Gender	0.096	0.135	0.008	0.838

3.5 Parametric bootstrap

The relative contribution of each variable on the outcome was also evaluated using TMLE. Four variables for which the TMLE and the cTMLE estimates substantially differed were considered at risk of positivity assumption violation: gender, history of musculoskeletal disease, of pulmonary disease and hypotension at hospital admission (Table 3).

3.6 Positivity bias

Table 4 summarizes the bias related to positivity violation for the four variables as determined by the parametric bootstrap as described above (again, we note since the data-generating distribution in this case is known, we can directly determine the true value of the parameter). As expected, the variable for which the difference in the estimates was the most pronounced is the one where the TMLE was associated with the largest positivity bias: history of pulmonary disease (positivity bias = 0.369). The cTMLE estimator was associated with substantially less positivity bias as compared to TMLE. For instance, the positivity bias decreased from 0.369 to 0.001 for history of pulmonary disease.

3.7 Coverage of the 95% CI

Consistently, the variable with the largest positivity bias was also associated with the smallest 95% CI coverage (history of pulmonary disease: 0.055) (Table 4). As expected, the positivity bias reduction achieved with cTMLE lead to larger coverage of the 95% CI (history of pulmonary disease: 0.766). However, it was still below the nominal value of 95%. To assess whether this lack of coverage was due to the influence curve-based variance estimator and/or to the remaining amount

Table 5. 95% CI coverage and bias-corrected 95%CI coverage for cTMLE estimates.

	Coverage	Bias corrected Coverage
Gender	0.838	0.946
Musculoskeletal disease	0.782	0.947
Pulmonary disease	0.766	0.949
Hypotension	0.833	0.947

of bias, we computed the bias corrected coverage as describe above. As reported in Table 5, for the four variables, bias correction lead to coverage values close to the expected 95%, suggesting that the influence curve based variance estimator for cTMLE is valid in this context. In any case, the automated variable selection method and use of cross-validation for selecting model for clever covariate shows much better performance in estimation and inference.

4 Discussion

Based on a prospectively collected dataset of patients suffering from TBI (12), we were able to show that TMLE for variable importance measure is associated with substantial bias when the positivity assumption is not fully fulfilled. In this context, the use of cTMLE was associated with substantial bias reduction and better coverage.

These results are in line with the underlying theory. Indeed, the targeting step of TMLE relies on a clever covariate, which is a function of the inverse of the propensity score. Therefore, when the estimated propensity score is close to zero or one (i.e. when the practical positivity assumption is nearly violated), the clever covariate can blow up and cause great instability in the targeting step. Hence, despite nice asymptotic properties such as consistency, linearity and double robustness, TMLE estimators may be biased in finite samples when the positivity assumption is nearly violated. For five variables, we were able to show using parametric bootstrap that the positivity-related bias was substantial, resulting in coverage of the 95% CI close to zero. cTMLE represents a further advance over standard TMLE.[10] Previous work by Gruber et al.[10,11] have demonstrated that the collaborative targeted maximum likelihood estimator is asymptotically linear and consistent even when Q and g are both misspecified, providing that g solves a specified score equation implied by the difference between the Q and the true Q_0. This marks an improvement over the current definition of double robustness in the estimating equation literature, and specifically over standard targeted maximum likelihood estimators. Our results emphasize that this properties are particularly interesting when dealing with causal parameters and positivity issues. In this situation, likelihood-based cross-validation targeting the best estimator among all candidate TMLE estimators of Q_0 guaranties to avoid the inclusion in the model for g, any explanatory variable for which there is no contrast in A. Hence, choosing the best g, i.e. the one associated with the most consistent estimate for our parameter of interest, will in turn limit the impact of positivity violation. We were able to confirm these theoretical results by quantifying the positivity bias using parametric bootstrap. Consistently, cTMLE was less prone to positivity bias than TMLE.

These results were reinforced by the clinical findings obtained with cTMLE, which are in line with the known pathophysiology and consistent with those previously published concerning the

prognosis of mild TBI patients. We are able to identify several risk factors associated with the three-month post-injury GOSE. First, certain components of the patient medical history may contribute to functional disability at three months post-injury. It is indeed not surprising to find that psychiatric history is associated with a poorer three-month GOSE. Consistently with our results, Ponsford et al.[35] report that prior history of psychiatric disorders is associated with post-concussive symptoms three months after mild TBI. Interestingly, history of hepatic disease was found to be associated with worse prognosis. This may be, at least partially, explained by the coagulation abnormalities frequently associated with liver diseases and the higher incidence of liver disease in chronic alcoholism. We also looked at the relationship between the three-month post-injury GOSE and the genotypic polymorphisms ANKK1 (rs1800497) and ApoE. The T allele of rs1800497 has indeed been implicated in addiction disorders and has also been reported to be a risk factor for depression, childhood behavior and learning problems.[36] Interestingly, veterans with PTSD who carried the T allele had more symptoms of anxiety, depression and social dysfunction than C/C homozygotes.[37]

Some limitations should be highlighted. First, the cTMLE-based VIM as implemented in the present study required to dichotomize the covariates. However, some extensions have been developed to pursue VIM for continuous variables.[38,39] Second, although derived from causal inference, the parameters estimated for VIM using TMLE or cTMLE may only be interpreted causal if the usual underlying causal assumptions hold. In the context of VIM, this may be true for some variables but not for others. Third, despite substantial decrease in positivity bias with cTMLE, the coverage of the 95% confidence intervals were still far from the nominal value in case of strong positivity bias. Finally, it should be emphasized that computational feasibility may sometimes be an issue with cTMLE. Multicore parallel computing is strongly recommended to speed up the procedure when analyzing large dataset and wealthy Super Learner library.

This study confirms that cTMLE holds promise as a fully automated procedure that can harness the power of any machine learning algorithm, to return estimates of variable importance optimized for specific clinically relevant parameters. It has the power to reduce the arbitrariness of typical statistical exercises with high dimensional data, while not sacrificing the ability to target certain associations. Most importantly, these estimates will all derived from an automated procedure, a variable importance machine.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: All statements in this report, including its findings and conclusions, are solely those of the authors and do not necessarily represent the views of the Patient-Centered Outcomes Research Institute (PCORI), its Board of Governors or Methodology Committee.

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Short Communication

Plasma Anti-Glial Fibrillary Acidic Protein Autoantibody Levels during the Acute and Chronic Phases of Traumatic Brain Injury: A Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot Study

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Abstract

We described recently a subacute serum autoantibody response toward glial fibrillary acidic protein (GFAP) and its breakdown products 5–10 days after severe traumatic brain injury (TBI). Here, we expanded our anti-GFAP autoantibody (AutoAb[GFAP]) investigation to the multicenter observational study Transforming Research and Clinical Knowledge in TBI Pilot (TRACK-TBI Pilot) to cover the full spectrum of TBI (Glasgow Coma Scale 3–15) by using acute (<24 h) plasma samples from 196 patients with acute TBI admitted to three Level I trauma centers, and a second cohort of 21 participants with chronic TBI admitted to inpatient TBI rehabilitation. We find that acute patients self-reporting previous TBI with loss of consciousness (LOC) ($n=43$) had higher day 1 AutoAb[GFAP] (mean ± standard error: 9.11 ± 1.42; $n=43$) than healthy controls (2.90 ± 0.92; $n=16$; $p=0.032$) and acute patients reporting no previous TBI (2.97 ± 0.37; $n=106$; $p<0.001$), but not acute patients reporting previous TBI without LOC (8.01 ± 1.80; $n=47$; $p=0.906$). These data suggest that while exposure to TBI may trigger the AutoAb[GFAP] response, circulating antibodies are elevated specifically in acute TBI patients with a history of TBI. AutoAb[GFAP] levels for participants with chronic TBI (average post-TBI time 176 days or 6.21 months) were also significantly higher (15.08 ± 2.82; $n=21$) than healthy controls ($p<0.001$). These data suggest a persistent upregulation of the autoimmune response to specific brain antigen(s) in the subacute to chronic phase after TBI, as well as after repeated TBI insults. Hence, AutoAb[GFAP] may be a sensitive assay to study the dynamic interactions between post-injury brain and patient-specific autoimmune responses across acute and chronic settings after TBI.

Key words: autoantibody; autoimmunity; biomarkers; glia; traumatic brain injury

Introduction

Traumatic brain injury (TBI) causes transient opening of the brain–blood barrier, which is often followed by neural cell damage or death. During the acute phase of TBI, a number of brain-specific proteins are released into the cerebrospinal fluid and/or blood (serum/plasma). A partial list includes neuronal proteins (ubiquitin-C-terminal hydrolase-L1 ([UCH-L1]), microtubule associated protein tau (MAPT/Tau), neuron specific enolase (NSE), axonal proteins (neurofilament-H, αII-spectrin breakdown products

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[SBDPs]), dendritic protein (MAP2), glial proteins (glial fibrillary acidic protein [GFAP], S100β) oligodendrocyte proteins (myelin basic protein [MBP]), and endothelial cell derived proteins (e.g. von Willebrand factor [VWF]).[1–4]

Because the brain is a site of immune-privilege, most of these proteins are not generally accessible to the immune system. TBI represents a situation where high concentrations of brain proteins are transiently released into the circulation and become accessible to the immune system.

Previous reports have documented brain-directed autoimmunity in neurological and neurodegenerative diseases such as Alzheimer disease, stroke, epilepsy, spinal cord injury, and paraneoplastic syndromes.[5–11] In human TBI, however, autoimmunity has only been examined in a limited way and focused on autoantibodies against preselected antigens such as MBP, S100β, and glutamate receptors.[12–18] Among investigators in the areas of autoimmunity and biomarkers,[19–23] Tanriverdi and associates[20] showed the presence of antipituitary antibodies in patients serum 3 years after head trauma. In other investigations,[24,25] Marchi and colleagues[25] demonstrated that antiglial protein S100β autoantibody levels are elevated in football players with repeated concussions. In parallel, we recently reported a rather unexpected immunodominant autoantibody response to GFAP and its breakdown products (BDPs) in a subset of patients with severe TBI.[26]

Based on our previous anti-GFAP-autoantibody study,[26] we observed that GFAP appeared to be a dominant brain-derived autoantigen after severe TBI. We hypothesized that TBI causes protease-mediated GFAP-BDP formation in injured glial cells. This is followed by the subsequent release of GFAP-BDPs in substantive quantity through a compromised brain–blood barrier into the circulation.[24,27–29] This combination allows GFAP and GFAP-BDPs to become accessible to and recognized by the immune cells as nonself-proteins, triggering autoantibody response in those individuals.

While GFAP is an intracellular antigen and the central nervous system (CNS) is normally considered immune-privileged, it is still conceivable that autoantibodies can gain assess to the CNS tissue where such an antigen is localized. For example MBP, myelin oligodendrocyte glycoprotein (MOG), and other intracellular myelin proteins in the spinal cord appear to be attacked by the immune system in multiple sclerosis and other demyelination diseases.[30] Hence, it is possible that an autoantibody specifically targeting a major brain protein such as GFAP might trigger a persistent autoimmune activation, which could negatively impact on long-term recovery from TBI.

Thus, we sought to expand our anti-GFAP autoantibody (AutoAb[GFAP]) investigation to the Transforming Research and Clinical Knowledge in TBI Pilot (TRACK-TBI pilot) study,[31] a multicenter observational study that covers the full spectrum of TBI (Glasgow Coma Scale [GCS] 3–15) with acute (<24 h) plasma samples available from 196 patients with acute TBI admitted to Level I trauma centers, as well as a second cohort of 21 participants with chronic TBI admitted to an inpatient rehabilitation center.

METHODS

TBI patients

Patients with acute TBI were identified and recruited on arrival at one of three Level I trauma centers and one inpatient TBI rehabilitation center as part of the multicenter prospective TRACK-TBI pilot study.[31] Study protocols were approved by the Institutional Review Boards of participating centers—acute sites: San Francisco General Hospital (SFGH); University of Pittsburgh Medical Center (UPMC), University Medical Center Brackenridge (UMCB); rehabilitation site: Mount Sinai Rehabilitation Center (MSRC). All participants or their legal authorized representatives provided written informed consent. At follow-up outcome time points, consent from participants from whom previous consent was obtained from a legally authorized representative was obtained for continuation in the study if the patient was neurologically improved to be capable of self-consent.

To be eligible for the TRACK-TBI pilot study, patients with acute TBI presented within 24 h of injury to the emergency department and had a history of trauma to the head sufficient to triage to noncontrast head computed tomography (CT) scan using the American College of Emergency Physicians/Centers for Disease Control evidence-based joint practice guideline, while patients with chronic TBI had sufficient neurologic impairment to triage to inpatient TBI rehabilitation.

Details of loss of consciousness (LOC), amnesia, and source of trauma were recorded on screening, and informed consent was obtained. GCS score was assessed by a neurosurgeon at admission and was reconfirmed by study personnel at the time of biomarker collection. For those with chronic TBI, plasma samples were collected on presentation to rehabilitation at MSMC with an average post-injury time of 188 days (6.2 months). We further identified patients with acute TBI with self-reported previous TBI with or without LOC (Table 1).

Biosample collection

Blood samples were collected from patients with acute TBI who consented to genetic and proteomic analysis within 24 h of injury ($n = 196$). Blood samples from those with chronic TBI were collected at the indicated time point. Plasma was extracted as supernatant after centrifugation of whole blood in ethylenediaminetetracetic acid (EDTA) blood tubes for 5–7 min at 4000 rpm according to the National Institutes of Health/National Institute of Neurological Disorders and Stroke TBI Common Data Elements Biospecimens and Biomarkers Working Group recommendations.[32] In addition, 16 commercial control plasma samples collected with EDTA blood tubes (Bioreclamation Inc., mean ± standard deviation [SD] 39.1 ± 17.2 years old) were age-matched with the acute ($n = 196$; 42.1 ± 18.1 years old) and chronic TBI ($n = 21$; age 44.4 ± 20.5 years old) samples and assayed for AutoAb[GFAP].

Measurement of AutoAb[GFAP]

To detect and quantify AutoAb[GFAP] levels in biosamples, we used our previously published manifold autoantibody immunoblotting assay format[26] (see Supplementary Fig. 1 for assay set-up; see online supplementary material at ftp.liebertpub.com). Briefly, human brain GFAP protein or human brain fraction enriched in GFAP protein (20 μg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis on 4–20% Tris-glycine 1-well gel and electrotransferred to polyvinylidene fluoride (PVDF) membrane. PVDF membranes were then clamped into the Mini-Protean II Multiscreen apparatus (Bio-Rad), and individual lanes were blocked and probed with human sera diluted at 1:100, unless otherwise noted.

This manifold autoantibody immunoblot assay[26] requires only a 1/100 dilution (e.g., 1 μL in 100 μL). We serially diluted the plasma to verify that the signal is plasma concentration-dependent provided that it is within the optical density (OD) readings for the

Table 1. Demographics and Injury Characteristics of Patients with Traumatic Brain Injury

	Acute TBI	*Chronic TBI**
Age	N = 196	N = 21
Mean, SD	42.4, 17.8	44.4, 20.5
Range	16–86	19–81
Sex	N = 196	N = 21
Male	151 (73%)	16 (76%)
Female	55 (27%)	5 (24%)
GCS	N = 196	N = 21
3–8	12 (6%)	—
9–12	6 (3%)	—
13–15	160 (82%)	—
Unknown	18 (9%)	—
Previous TBI	N = 196	N = 21
None	106 (54%)	4 (19%)
Yes, without LOC	47 (24%)	4 (19%)
Yes, with LOC	43 (22%)	13 (62%)
Admission Head CT	N = 196	N = 21
Negative	108 (55%)	—
Extra-axial only	22 (11%)	5 (24%)
Intra-axial only	24 (12%)	3 (14%)
Extra + Intra-axial	42 (21%)	3 (14%)
Unknown	—	10 (48%)
Outcome (6-month)	N = 137	N = 17
GOS-E = 1	7 (5%)	0 (0%)
GOS-E = 2	1 (1%)	0 (0%)
GOS-E = 3	10 (7%)	1 (5%)
GOS-E = 4	4 (2%)	5 (24%)
GOS-E = 5	5 (10%)	5 (24%)
GOS-E = 6	21 (15%)	1 (5%)
GOS-E = 7	39 (28%)	1 (5%)
GOS-E = 8	44 (32%)	4 (19%)

TBI, traumatic brain injury; SD, standard deviation; GCS, Glasgow Coma Scale; LOC, loss of consciousness; CT, computed tomography; GOS-E, Glasgow Outcome Scale-Extended,

*GCS data was unavailable for chronic TBI patients. CT pathology was positive for all chronic TBI patients with CT data.

spectrometer. Secondary antibodies used were either alkaline phosphatase (AP)-conjugated goat antihuman immunoglobulin G (IgG) or AP-conjugated donkey antihuman IgG diluted 1:10,000 (Jackson ImmunoResearch).

Blots were developed at room temperature with substrate 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt and nitro-blue tetrazolium chloride solution for 10 min. We also routinely performed in-solution pre-absorption with GFAP protein (2 μg/100 μL) as a control study. The bands of interest on the blotting membrane disappear after pre-absorption (data not shown). Quantification of autoantibody reactivity on immunoblots was performed via computer-assisted densitometric scanning (Epson 8836XL high-resolution scanner and NIH Image J densitometry software). Autoantibody levels were expressed in arbitrary densitometry units.

Values are reported as mean and standard error (SE) unless stated otherwise. Analysis of variance (ANOVA) was used for multigroup analysis; Tukey *post hoc* test used to assess mean differences between subgroups as well as distinguish homogeneous subsets. Statistical significance was assessed at $p < 0.05$. Statistics were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) and Statistical Analysis System (SAS) version 9.2, (SAS Institute, Inc., Cary, NC) unless stated otherwise.

Results

Anti-GFAP autoantibody levels in acute plasma samples from TRACK-TBI pilot study

Because we previously identified a dominant autoantibody response to glial intermediate filament protein GFAP among patients with severe TBI,[26,33–35] here we sought to expand these findings by using the TRACK-TBI pilot study cohorts and plasma samples.[1,36,37] Of 586 subjects with acute TBI from the TRACK-TBI pilot, we identified 196 with available acute plasma samples (collected within 24 h of injury) for this autoantibody study. Study patients covered the range of initial GCS of 3–15, which are reported with age, sex, and admission head CT distributions in Table 1. The TRACK-TBI pilot study also recorded self-reported previous TBI history (apart from the index TBI of enrollment), with the following categories: no previous TBI ($n = 106$), previous TBI without LOC ($n = 47$), and previous TBI with LOC ($n = 43$) (Table 1).

Autoantibodies reacting with intact GFAP (50 kDa) and its various BDPs (48–38 kDa) were assayed using quantitative manifold immunblotting developed previously.[3,26] The distribution for AutoAb[GFAP] (mean ± SE) was 2.90 ± 0.92 units for healthy controls, 2.97 ± 0.37 units for patients with acute TBI reporting no previous TBI, 8.01 ± 1.80 units for patients with acute TBI reporting previous TBI without LOC, and 9.11 ± 1.42 units for patients with acute TBI reporting previous TBI with LOC.

ANOVA showed a significant difference across groups ($p < 0.001$); Tukey *post hoc* test demonstrated that healthy controls and patients with acute TBI reporting no previous TBI constituted a statistically different subgroup in AutoAb[GFAP] levels than patients with acute TBI reporting previous TBI either with or without LOC (Fig. 1). Specifically, patients with acute TBI reporting previous TBI with LOC showed significantly elevated AutoAb[GFAP] levels than healthy controls (mean increase 6.21 ± 2.26, $p = 0.032$) and patients reporting no previous TBI (mean increase 6.14 ± $p < 0.001$), but not with patients with acute TBI reporting previous TBI without LOC (mean increase 1.10 ± 1.62, $p = 0.906$); patients with acute TBI reporting previous TBI without LOC showed significantly elevated AutoAb[GFAP] levels

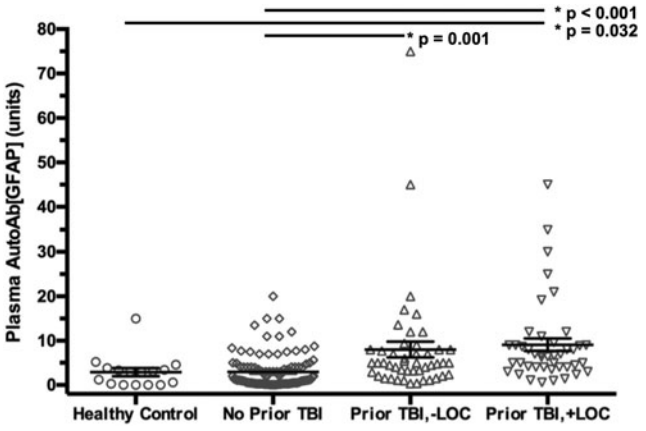

FIG. 1. Mean and standard error of the mean are shown for each respective patient subgroup (healthy control, acute traumatic brain injury [TBI] reporting no prior TBI, acute TBI reporting prior TBI without loss of consciousness [LOC], acute TBI reporting prior TBI with LOC). The plasma AutoAb[GFAP] (glial fibrillary acidic protein autoantibody) is shown in units as described in the Methods section of the article. Statistically significant differences across subgroups are denoted with (*) and the respective p value.

when compared with patients with acute TBI reporting no previous TBI (mean increase 5.04 ± 1.35, $p = 0.001$), but not with healthy controls (mean increase 5.11 ± 2.23, $p = 0.103$). No difference was observed in AutoAb[GFAP] between healthy controls and patients with acute TBI reporting no previous TBI (mean increase 0.07 ± 2.07, $p = 0.999$).

In previous reports, we have assayed the same patient plasma samples for GFAP (and its BDP) levels.[36,37] Thus, we examined whether there is a correlation between GFAP antigen levels and AutoAb[GFAP] levels in these samples. As expected, we did not find a correlation between the two (data not shown).

In addition, we sought to examine acute AutoAb[GFAP] distributions across different initial GCS scores. Because of the relatively small number of samples for those with lower GCS, by convention we grouped acute patients to three GCS categories for autoantibody comparison purposes: GCS 3–8 ($n = 12$; mean ± SE: 3.35 ± 0.87), GCS 9–12 ($n = 6$, 4.37 ± 1.59), GCS 13–15 ($n = 169$, 6.16 ± 0.72). Results on ANOVA showed no statistically significant differences in acute AutoAb[GFAP] levels across the three GCS categories ($p = 0.197$).

We also examined acute AutoAb[GFAP] distributions by presence of intracranial pathology on admission head CT, across categories of ''no intracranial pathology'' ($n = 108$), ''extra-axial pathology only'' ($n = 22$), ''intra-axial pathology only'' ($n = 24$), and ''both extra-axial and intra-axial pathology'' ($n = 42$). Results on ANOVA showed no statistically significant differences in acute AutoAb[GFAP] levels across the four categories (mean ± SE: 6.48 ± 0.87; 5.02 ± 2.01; 5.72 ± 2.04; 3.22 ± 0.49, respectively; $p = 0.197$).

To further explore the relationship between pathological injury severity and AutoAb[GFAP], we analyzed the distribution of AutoAb[GFAP] across Marshall CT categories. Because of the small numbers of individual Marshall CT scores of 3 ($n = 9$), 4 ($n = 2$), 5 ($n = 12$), and 6 ($n = 1$), we combined Marshall score 3–6 into a single category ''3+''. AutoAb[GFAP] distributions were as follows: Marshall 1 ($n = 96$, mean ± SE: 6.20 ± 0.91), Marshall 2 ($n = 78$, 5.57 ± 0.96), Marshall 3+ ($n = 22$, 2.47 ± 0.64) and showed no statistically significant differences across Marshall CT categories ($p = 0.148$).

Anti-GFAP autoantibody levels in chronic plasma samples from TRACK-TBI pilot study

We previously demonstrated that post-TBI serum AutoAb[GFAP] shows a delayed increase, beginning about 5–6 days after severe TBI and sustained to at least 10 days.[26,38] Here, we examined AutoAb[GFAP] levels in chronic TBI plasma samples collected from 21 subjects during rehabilitation. The demographics of these subjects are tabulated in Table 1. Initial GCS and CT Marshall scores or Glasgow Outcome Score-Extended data were unavailable for patients with chronic TBI.

All patients triaged to the rehabilitation facility were assessed with an index injury severe enough to warrant inpatient rehabilitation, with Rancho Los Amigos-Revised (RLA) score distributions of the following on admission to the rehabilitation facility: RLA 1 (No Response, Total Assistance, n = 1); RLA 2 (Generalized Response, Total Assistance, $n = 2$), RLA 3 (Localized Response, Total Assistance, n = 2), RLA 4 (Confused/Agitated, Maximal Assistance, $n = 1$), RLA 5 (Confused, Inappropriate/Nonagitated, Maximal Assistance, $n = 6$), RLA 6 (Confused, Appropriate, Moderate Assistance, $n = 4$), RLA 7 (Automatic, Appropriate, Minimal Assistance for Activities of Daily Living (ADL), $n = 1$), RLA 8 (Purposeful, Appropriate, Stand-By Assistance, $n = 0$), RLA 9 (Purposeful, Appropriate, With Standby Assist on Request, $n = 0$), RLA 10 (Purposeful, Appropriate, Modified Independent, $n = 0$), RLA Unknown, ($n = 5$).

Thus, all patients with chronic TBI with known RLA had a score of 7 or less, with 16 (76%) of the 21 total patients needing moderate assistance for ADL because of their brain injury (5 [24%] needing total assistance, 7 [33%] needing maximal assistance, and 4 [19%] needing moderate assistance). Hence, we observe that the chronic TBI population in this study is one of overall moderate to total impairment in ADL. CT data were available for 11 of 21 patients with chronic TBI (5 extra-axial hemorrhage only, 3 intra-axial hemorrhage only, 3 both extra- and intra-axial hemorrhage).

The post-injury time ranged 16–250 days after injury, with an average of 176.4 days (or 6.4 months) post-injury (Table 2). Using ANOVA, we show that the AutoAb[GFAP] levels were significantly elevated in patients with chronic TBI (mean 15.08 ± 2.82 units, $p < 0.001$) compared with healthy controls as previously reported (mean 2.90 ± 0.93 units) (Fig. 2).

We also plotted a graph of the plasma AutoAb[GFAP] against the time post-injury based on this set of 21 patients. Each patient with chronic TBI only had one timed plasma sample drawn as part of the TRACK-TBI pilot study (Fig. 3); while the sample size is limited, no significant correlation was found between post-injury time and AutoAb[GFAP] levels (Spearman rank correlation test, data not shown).

We also examined the relationship between CT intracranial lesion and AutoAb[GFAP] levels in these patients with chronic TBI. Results on ANOVA showed no statistically significant differences across the four categories (mean ± SE): extra-axial only, 14.32 ± 6.01; intra-axial only, 8.27 ± 2.88; both extra- and intra-axial, 13.82 ± 7.98, unknown CT pathology, 13.07 ± 3.86; $p = 0.168$.

Discussion

In this study, we expand on our previous finding that there is a dominant anti-GFAP autoantibody response within 5–10 days among a subset of patients with severe TBI.[26,39] While the number of plasma samples is still relatively small within the cohort, the TRACK-TBI pilot dataset was selected for this study because it is well-characterized with 13 published articles regarding various components of these TBI patients across the full range of TBI severity—including proteomic and genetic biomarkers, neuroimaging, and outcome data.[31,36,37,40–43]

Based on the 217 subjects with available biosamples from this cohort, we identified that anti-GFAP autoantibody levels were elevated in acute plasma samples from brain injury subjects who had a self-reported history of previous TBI with or without LOC when compared with patients with acute TBI without self-reported previous TBI (Fig. 1). There is no correlation between GFAP antigen levels and GFAP autoantibody levels in these acute samples and to initial GCS.

We also found no statistically significant differences between AutoAb[GFAP] and acute CT pathology—widely used as the current clinical standard for TBI diagnosis and a surrogate marker of brain injury after acute TBI.[1,44–46] Because newly acquired anti-GFAP antibody response usually takes about 5 days to manifest,[26,47,48] it is unlikely that the acute post-TBI autoantibody levels we report here were from a *de novo* response to current TBI, but rather to a sustained increase because of previous head injuries. At present, however, we cannot rule out whether the acute TBI event might serve to be an antigen-boosting event for those with pre-existing anti-GFAP antibody titers. It is also interesting to consider that repeated mild TBI/concussion can potentially serve as an autoantigen-boosting event.

Table 2. Plasma Glial Fibrillary Acidic Protein Autoantibody Levels in Patients with Traumatic Brain Injury*

		Acute TBI			*Chronic TBI*	
Post-injury time		*N = 196*			*N = 21*	
Mean ± SD		10.6 ± 6.3 (h)			176.4 ± 44.5 (days)	
Range		0.5 to 23.9 (h)			16.0 to 250.0 (days)	
GCS	*N*	*Mean (SE)*	*Sig. (p)*	*N*	*Mean (SE)*	*Sig. (p)*
3–8	12	3.35 (0.87)	0.136	—	—	—
9–12	6	4.37 (1.59)		—	—	
13–15	120	6.16 (0.72)		—	—	
Unknown	18	1.73 (2.35)		—	—	
Previous TBI	*N*	*Mean (SE)*	*Sig. (p)*	*N*	*Mean (SE)*	*Sig. (p)*
None	106	2.97 (0.37) [a]	<0.001	4	13.25 (2.43)	0.956
Yes, without LOC	47	8.01 (1.80) [b]		4	15.41 (6.56)	
Yes, with LOC	43	9.11 (1.42) [b]		13	15.54 (4.18)	
Admission head CT	*N*	*Mean (SE)*	*Sig. (p)*	*N*	*Mean (SE)*	*Sig. (p)*
Negative	108	6.48 (0.87)	0.197	—	—	0.168
Extra-axial only	22	5.02 (2.01)		5	14.32 (6.01)	
Intra-axial only	24	5.72 (2.04)		3	8.27 (2.88)	
Extra- + intra-axial	42	3.22 (0.49)		3	13.82 (7.98)	
Unknown	—	—		10	13.07 (3.86)	

TBI, traumatic brain injury; SD, standard deviation; GCS, Glasgow Coma Scale; SE, standard error of the mean; LOC, loss of consciousness; CT, computed tomography;.

*Blood draw for GFAP-AutoAb post-injury time calculated from time of injury. GCS data were unavailable for patients with chronic TBI. CT pathology was positive for all patients with chronic TBI with CT data. [a] and [b] denote statistically significant subgroups on the Tukey *post hoc* test.

Our study is the first to report AutoAb[GFAP] values across the spectrum of acute TBI. The reason for anti-GFAP reactivity in a subset of healthy controls is not completely known. We have reported similar results in our first study on AutoAb[GFAP].[26] We also noted that autoantibodies to other human autoantigens have been reported in normal populations.[49,50] We suspect that the baseline anti-GFAP autoantibody levels we observed in certain healthy controls likely reflect the TBI health history of those subjects—e.g., they may have experienced previous unreported concussions or other subclinical neurological events.[25]

It is also presently unclear as to why AutoAb[GFAP] was statistically significantly elevated in those with an acute TBI and history of previous TBI when compared with those with acute TBI without a previous history of TBI, but not healthy controls. The samples captured from the auto rehabilitation cohort with confirmed previous TBI, however, did demonstrate statistically higher GFAP autoantibody levels. Whether this contradiction is reflective of the small sample size, a high prevalence of unreported TBI in the control group, and/or a combination thereof remains to be determined.

It is also possible that the GFAP autoantibody level represents not only initial injury severity/mortality, but also individual variability in the immune response and/or clearance of autoantibodies. Hence, our study should be considered preliminary and future studies with serial collection of GFAP autoantibodies are therefore needed to better quantitate the time course in individuals to better characterize the hypothesized variability.

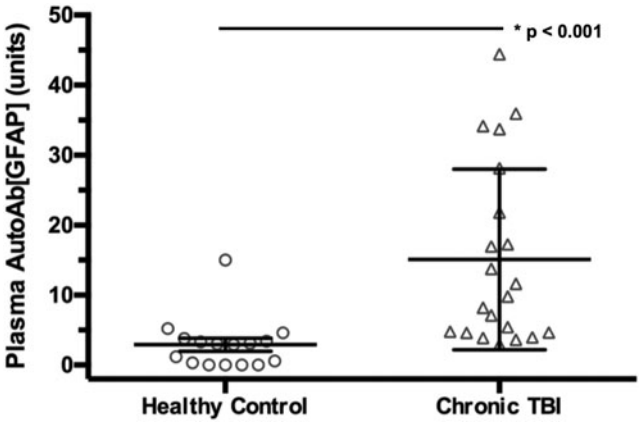

FIG. 2. Mean and standard error of the mean are shown for healthy control versus patients with chronic traumatic brain injury (TBI). The plasma AutoAb[GFAP] (glial fibrillary acidic protein autoantibody) is shown in units as described in the Methods section of the article. Statistically significant differences across subgroups are denoted with (*) and the respective *p* value.

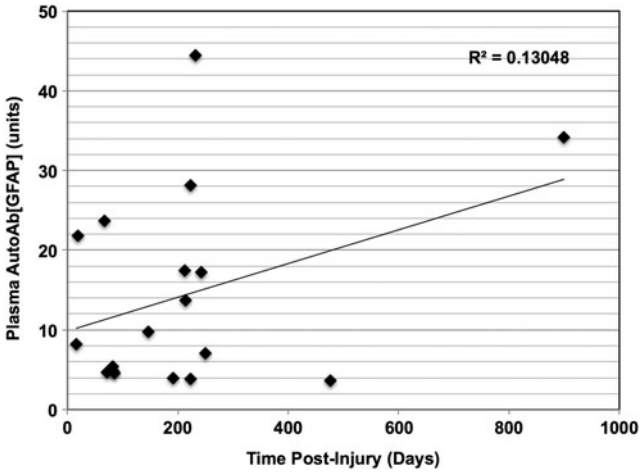

FIG. 3. Scatterplot for plasma AutoAb[GFAP] (glial fibrillary acidic protein auto-antibody) plotted against time post-injury for 21 patients with chronic traumatic brain injury (TBI). The plasma AutoAb[GFAP] is shown in units as described in the Methods section of the article. The correlation coefficient (R^2) is shown.

All samples were collected within 24 h after the current TBI event and thus the plasma AutoAb[GFAP] we measured in these patients with acute TBI likely reflects previous brain injury or perturbation incidents. Patients reporting previous TBI without LOC had a slightly lower AutoAb[GFAP] level on average than those reporting previous TBI with LOC. This preliminarily suggests that the severity of previous exposure exerts some effect on the magnitude of the AutoAb[GFAP] response measureable in plasma. Future studies with a larger population of post-TBI patients in which initial injury characteristics are available is needed to further validate this finding, however.

While preliminary, this is the first report of significant plasma AutoAb[GFAP] elevation in patients with TBI at the chronic time point (mean >6 month) compared with age-matched controls (Fig. 2). Because the autoantibody response is a marker of sustained immunological memory, it may be the case that neuronal or glial autoantibody biomarkers can be useful to confirm a diagnosis of chronic TBI in cases where history is vague or incomplete.

Some of the limitations of the current study are as follows. Currently, we focused on IgG responses; in future studies, we plan to examine in parallel IgM-based autoantibody responses to investigate acute changes. To increase the throughput of the anti-GFAP autoantibody assays, it will be desirable to use microplate-based enzyme-linked immunosorbent assays; we are working toward this direction. In addition, because of institutional-specific differences in medical record documentation, all previous injury information was patient-reported and additional injury characteristics (e.g., acute GCS in the rehabilitation setting) could not be independently confirmed and/or clarified.

Another limitation is the lack of longitudinal blood samples within the same patient, and thus we were unable to follow the temporal profile of AutoAb[GFAP] response. To this end, we will be expanding our AutoAb[GFAP] studies to the ongoing, NIH-funded prospective multicenter TRACK-TBI study[51] with acute (day 1, 3, 5), subacute (2 weeks), and chronic (6 month) blood samples from up to 2700 patients with TBI across injury severities, as well as 300 non-TBI controls, as part of the U.S. Department of Defense TBI Endpoints Development Initiative.[52] Data from these future studies will allow us to examine whether elevations of post-injury AutoAb[GFAP] associate with patient outcome.

Conclusion

AutoAb[GFAP] assays may be useful to study the dynamic interactions among brain autoimmune mechanisms post-TBI across acute and chronic injury settings. There are two important new findings reported in this study: (1) We find that in the setting of acute TBI, plasma AutoAb[GFAP] levels associate with a history of past exposure to TBI; (2) Further, this is the first study to report elevated AutoAb[GFAP] levels at a chronic time point (average of 6 months post-injury) among patients with moderate to severe TBI. With emerging attention on reexamining TBI as a chronic condition with various comorbidities,[3,38,53–56] we can now add brain protein-targeting autoantibodies to a growing list of potential useful biomarkers for studying at-risk acute and chronic TBI populations.

Acknowledgments

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Author Disclosure Statement

K.K.W. holds stocks of Banyan Biomarkers, Inc., a company interested in commercialization of diagnostic tests for TBI. For the remaining authors, no competing financial interests exist.

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COMT Val^{158}*Met* polymorphism is associated with nonverbal cognition following mild traumatic brain injury

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Abstract

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The TRACK-TBI Investigators are listed in the Appendix in alphabetical order by last name

Ethan A. Winkler and John K. Yue contributed equally to this work.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Mild traumatic brain injury (mTBI) results in variable clinical outcomes, which may be influenced by genetic variation. A single-nucleotide polymorphism in catechol-o-methyltransferase (*COMT*), an enzyme which degrades catecholamine neurotransmitters, may influence cognitive deficits following moderate and/or severe head trauma. However, this has been disputed, and its role in mTBI has not been studied. Here, we utilize the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) study to investigate whether the *COMT* $Val^{158}Met$ polymorphism influences outcome on a cognitive battery 6 months following mTBI—Wechsler Adult Intelligence Test Processing Speed Index Composite Score (WAIS-PSI), Trail Making Test (TMT) Trail B minus Trail A time, and California Verbal Learning Test, Second Edition Trial 1–5 Standard Score (CVLT-II). All patients had an emergency department Glasgow Coma Scale (GCS) of 13–15, no acute intracranial pathology on head CT, and no polytrauma as defined by an Abbreviated Injury Scale (AIS) score of ≥3 in any extracranial region. Results in 100 subjects aged 40.9 (SD 15.2) years ($COMT\ Met^{158}/Met^{158}$ 29 %, Met^{158}/Val^{158} 47 %, Val^{158}/Val^{158} 24 %) show that the $COMT\ Met^{158}$ allele (mean 101.6±SE 2.1) associates with higher nonverbal processing speed on the WAIS-PSI when compared to Val^{158}/Val^{158} homozygotes (93.8±SE 3.0) after controlling for demographics and injury severity (mean increase 7.9 points, 95 % CI [1.4 to 14.3], p=0.017). The $COMT\ Val^{158}Met$ polymorphism did not associate with mental flexibility on the TMT or with verbal learning on the CVLT-II. Hence, $COMT\ Val^{158}Met$ may preferentially modulate nonverbal cognition following uncomplicated mTBI.

Keywords

Traumatic brain injury; Genetic factors; Cognitive function; Outcome measures; Human studies

Introduction

Traumatic brain injury (TBI)—defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force—is a comparatively common insult with variable outcomes [1, 2]. In the USA alone, at least 2.5 million people suffer TBIs annually [3], and it has been estimated that up to 5.3 million people are currently living with TBI-related disability [4]. TBI is frequently subdivided on the basis of injury severity into severe, moderate, and mild injury categories as defined by a Glasgow Coma Scale (GCS) score of 8 or less, 9-to-12, or 13-to-15, respectively [5, 6]. Although more severe injuries may disproportionately contribute to disability, the vast majority—70 to 90 %—of all TBI is characterized as “mild TBI” (mTBI) [7]. Within mTBI, considerable variability in outcome exists across individuals. Most make a complete recovery following mTBI [8, 9]; however, up to 20 % of patients experience persistent symptoms and/or cognitive or neuropsychiatric deficits [10]. Individuals with nearly identical injuries often manifest different symptoms, follow different clinical trajectories, and/or have varied functional outcomes [11]. Efforts are therefore needed to better identify those at greatest risk for posttraumatic sequela to better prognosticate and facilitate development of tailored therapy [1].

Studies have begun to investigate relationships between genetic variants within a number of candidate genes and outcome following TBI in an effort to elucidate such variability. One

form of this variance—called single nucleotide polymorphisms (SNPs)—is comprised of single nucleotide substitutions arising within a gene's coding sequence and/or regulatory elements which may influence either protein structure/function or abundance, respectively. Numerous polymorphisms have been identified [12–14], but those arising within genes encoding important proteins underlying neurotransmission are thought to play an influential role in the preservation and/or impairment in cognition following TBI [15]. Catechol-*O*-methyltransferase (COMT; encoded by the gene *COMT* on chromosome 22q11.2) represents one such molecule [16–18] and is an enzyme which inactivates catecholamine neurotransmitters, e.g., dopamine (DA), epinephrine, and norepinephrine, through 3-*O*-methylation of the benzene ring [19]. In brain regions important to cognition, e.g., the prefrontal cortex (PFC), low expression of DA reuptake transporters makes COMT inactivation the predominant regulator of dopaminergic synaptic transmission [19–21].

A relatively common SNP arising within the coding sequence at codon 158—known as *COMT* $Val^{158}Met$ (*rs4680*)—results in substitution of a methionine for valine at this position [19]. This substitution lessens the activity of COMT resulting in higher levels of dopamine in the PFC [22], and it has been shown that Val^{158}/Val^{158} individuals are up to four times more efficient at catabolizing catecholamines than Met^{158}/Met^{158} homozygotes [23]. In turn, higher bioavailability of catecholamines in the PFC in Met^{158}/Met^{158} subjects has been shown to confer a cognitive advantage over Val^{158}-carriers [24], and the Met^{158} allele is generally associated with an advantage in measures of memory, executive function, and tasks requiring attention [18, 25].

Cognitive symptoms, including memory loss, inattention, and impulsivity, are relatively common in TBI and are among the most debilitating consequences of TBI and may influence functional outcome [26]. A number of prior studies have suggested that disruption and/or dysregulation of dopaminergic transmission in the PFC may contribute to the pathogenesis of posttraumatic cognitive impairment [27]. Conversely, it has been suggested in other studies that the dopaminergic system may be pharmacologically targeted to ameliorate persistent cognitive deficits following TBI [28]. Despite its importance in modulating PFC neurotransmission, studies examining the relationship between the *COMT* $Val^{158}Met$ polymorphism and cognitive deficits following TBI have largely been equivocal [16–18]. To date, these studies have been limited to more severe injury, and whether the *COMT* $Val^{158}Met$ polymorphism influences posttraumatic cognitive deficits following mTBI has yet to be studied.

Here, we utilize the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) dataset, a database of demographic history, biomarkers, neuroimaging, and neuropsychiatric and neurocognitive outcomes obtained at three clinical sites [29], to evaluate whether the *COMT* $Val^{158}Met$ polymorphism influences cognitive performance 6 months following mTBI on a battery of three standardized tests—Wechsler Adult Intelligence Scale Fourth Edition Processing Speed Index subscale, Trail Making Test, and the California Verbal Learning Test Second Edition. We hypothesized that the *COMT* $Val^{158}Met$ polymorphism is associated with improved cognitive performance following mTBI. Our data demonstrates that the *COMT* $Val^{158}Met$ polymorphism associates with

cognitive performance in select domains, e.g., nonverbal processing speed, but not others, e.g., mental flexibility or verbal learning.

Materials and methods

Study design

The TRACK-TBI Pilot Study is a multicenter prospective observational study conducted at three Level 1 trauma centers in USA—San Francisco General Hospital, University of Pittsburgh Medical Center, and University Medical Center Brackenridge (UMCB) in Austin, Texas [29]—using the National Institutes of Health (NIH) and National Institute of Neurological Disorders and Stroke (NINDS) common data elements (CDEs) [30–33]. Inclusion criteria for the pilot study were adult patients presenting to a Level 1 trauma center with external force trauma to the head and clinically indicated head computed tomography (CT) scan within 24 h of injury. Exclusion criteria were pregnancy, comorbid life-threatening disease, incarceration, suicidal ideation/on psychiatric hold, and non-English speakers due to limitations in participation with outcome assessments. For the present study, our goal was to study the associations between *COMT Val*^{158}*Met* and cognition after isolated and uncomplicated mTBI. Therefore, our analysis was restricted to a subset of patients with a GCS ≥13, no skull fracture, or acute intracranial pathology—defined as the absence of intraparenchymal contusions or hemorrhage, intraventricular hemorrhage, epidural hematoma, acute subdural hematoma, or traumatic subarachnoid hemorrhage—on non-contrasted head CT within 24 h of injury, no polytrauma as defined by an Abbreviated Injury Scale (AIS) score ≥3 in any extracranial body region [34, 35], as well as no prior history of cerebrovascular accident or transient ischemic attack, brain tumor, schizophrenia, learning disability or developmental delay.

Eligible subjects were enrolled through convenience sampling at all three sites. Institutional review board approval was obtained at all participating sites. Informed consent was obtained for all subjects prior to enrollment in the study. For patients unable to provide consent due to their injury, consent was obtained from their legally authorized representative (LAR). Patients were then reconsented if cognitively able at later inpatient and/or outpatient follow-up assessments for continued participation in the study.

Biospecimen acquisition and genotyping

Specimen acquisition was performed as previously described [29]. In brief, blood samples for DNA genotyping analysis were collected via peripheral venipuncture or existing peripheral venous indwelling catheters within 24 h of injury. Samples were collected in BD Vacutainer K_2-EDTA vacutainer tubes, and subsequently aliquoted and frozen in cryotubes at −80 °C within 1 h of collection in accordance with recommendations from the NIH-CDE Biomarkers Working Group [Manley 2010]. DNA was extracted from isolated leukocytes using the Wizard® Genomic DNA Purification Kit as described by the manufacturer (Promega, Madison, WI) and reported in our previous work [36]. *COMT Val158Met* polymorphism (*rs4680*) was genotyped utilizing the TaqMan®SNP Genotyping Assay as described by the manufacturer (Applied Biosystems, Carlsbad, CA, Assay ID# C_25746809_50). For the purpose of evaluating a potential protective benefit of the *Met*^{158}

allele, Met^{158}/Met^{158} and Met^{158}/Val^{158} were combined as a single group as previously described for *COMT* [37–40] and other genetic polymorphisms in TBI [41–43]. Therefore, for data reporting and all figures, this group is referred to as Met^{158}.

Neuropsychiatric testing and outcome parameters

The NINDS defines measures of neuropsychological impairment as those "of neuropsychological functions, such as attention, memory, and executive function which are very sensitive to effects of TBI that affect everyday activities and social role participation [33]." To evaluate for neuropsychological impairment, all participants underwent outcome assessments at 6 months following TBI with a battery of NIH NINDS-designated "Core Measures"—those deemed most relevant and applicable across large TBI studies. For the current analysis, all three measures of the "Neuropsychological Impairment" domain of the outcome CDEs were included:

Wechsler Adult Intelligence Scale, fourth edition Processing Speed Index Subscale

The Wechsler Adult Intelligence Scale, fourth edition Processing Speed Index Subscale (WAIS-PSI) is a summary measure of nonverbal processing speed and is comprised of two non-verbal tasks (symbol search and coding) which require visual attention and motor speed [44]. In studies of TBI, it has been shown to predominately reflect impairment in perceptual processing speed with a small component attributable to working memory and only minimal contribution from motor speed [45]. The composite score is scalar, ranging from 50 to 150 to correspond to the 0.1st to 99.9th percentile of performance across age groups. Scores of ~90, 100, and ~110 correspond to the 25th, 50th, and 75th percentiles, respectively [44].

Trail Making Test

The Trail Making Test (TMT) is a two-part timed test (TMT-A and TMT-B), and both scores are measured in number of seconds needed for the patient to complete the task. TMT-A assesses visual processing, and TMT-B assesses mental flexibility and processing speed [46]. In order to derive a purer index of executive control and mental flexibility separate from visual processing and motor speed, we used the difference score between the Trial B and Trial A (TMT B-A) as previously described [47–49]. In this test, a lower score suggests improved performance.

California Verbal Learning Test, second edition

The California Verbal Learning Test, second edition (CVLT-II) is a verbal learning and memory task in which five learning trials, an interference trial, an immediate recall trial, and a post-20 min recall trial are performed. The CVLT-II trials 1–5 Standard Score is a summative score of the first five learning trials normed for age and sex and provides a global index of verbal learning ability [50]. The CVLT-II was substituted for the Rey Auditory Verbal Learning Test (RAVLT) listed in the NIH NINDS outcome CDEs due to relevant revisions of the second edition and higher consistency on between-norm sets [51].

Statistical analysis

Group differences in patient demographics and mechanism of injury across *COMT Met*^{158} carriers versus *Val*^{158}/*Val*^{158} homozygotes were assessed by Pearson's chi-squared test (X^2) for categorical variables and analysis of variance (ANOVA) for continuous variables. Fisher's exact test was used to assess for differences in categorical variables with group counts ≤5. Means and standard deviations are reported for continuous descriptive variables. Group differences are reported between *COMT* genotype and each outcome measure using ANOVA. Multivariable linear regression was performed for each of the three outcome measures to adjust for age and education years as recommended [44–46, 49, 50]; the WAIS-PSI Composite Score and CVLT-II trials 1–5 Standard Score are already age-normed and thus further adjusted only for education years, while the TMT B-A score was further adjusted for age and education years. As this is a study of mTBI, the GCS was used to adjust for injury severity (GCS 15 vs. less than 15). The adjusted unstandardized coefficient of regression (B) and associated standard error (SE) was used to quantify mean increase or decrease in the outcome measure associated with a per-unit increase in a continuous predictor or a change in the subcategory of a categorical predictor. All multivariable regression models conformed to tests for goodness-of-fit. To account for race stratification, race was entered onto the multivariable regression with three subcategories to include the two largest race categories (Caucasian, African-American/African) as well as a third category of aggregated "other races" for races with small (<5) group counts. Significance was assessed at α=0.05. All analyses were performed using Statistical Package for the Social Sciences (SPSS) v.22 (IBM Corporation, Chicago, IL). Figures were constructed with GraphPad Prism v.6 (GraphPad Software, La Jolla, CA).

Results

Patient demographics and mechanisms of injury

In total, the present study included 100 subjects (Table 1). Overall, subjects had a mean age of 40.9 years (SD 15.2) and were 66 % male. The race distribution was 70 % Caucasian, 14 % African American/African, 5 % Asian, 1 % American Indian/Alaskan Native, 1 % Hawaiian/Pacific Islander, and 9 % more than one race. Subjects had a mean of 14.2 years of education (SD 2.9). Mechanisms of injury were 33 % fall, 26 % motor vehicle crash, 22 % pedestrian versus auto, 15 % assault, and 4 % struck by/against object. GCS distribution was 3, 20, and 77 % for GCS of 13, 14, and 15, respectively. Distribution of admission GCS did not change with respect to genotype. For injury severity classification, GCS of 13 and 14 were combined into a single group of "GCS less than 15″. There was also no difference in posttraumatic amnesia—another important predictor for posttraumatic cognitive impairment—across genotypes [11, 52–54]. In total, 66 subjects were discharged from the emergency department (ED), 30 were admitted to the hospital ward, and 4 were admitted to the intensive care unit (ICU). No statistically significant difference in ED disposition was observed across genotypes (Table 1).

COMT genotype distribution was 29 % *Met*^{158}/*Met*^{158} (n= 29), 47 % *Met*^{158}/*Val*^{158} (n=47), and 24 % *Val*^{158}/*Val*^{158} (n= 24). *COMT* allelic frequencies (A=0.53, G=0.47) were not found to deviate significantly from Hardy-Weinberg equilibrium (X^2=0.33, p=0.566). Years

of education were higher for *Met*^{158} carriers than for *Val*^{158}/*Val*^{158} homozygotes (p=0.016), and a higher prevalence of *Val*^{158}/*Val*^{158} homozygotes was noted in African-American/African subjects (p=0.042). No other significant differences were observed in the distribution of each demographic and clinical descriptor across *COMT Met*^{158} and *Val*^{158}/*Val*^{158} genotypes (Table 1).

Outcome measures

We first assessed whether the *COMT Val*^{158}*Met* polymorphism was associated with divergent performance on three primary cognitive measures—WAIS-PSI, TMT B-A, and CVLT-II—following isolated, uncomplicated mTBI. *COMT Met*^{158} carriers showed significantly higher nonverbal processing speed on WAIS-PSI when compared to *COMT Val*^{158}/*Val*^{158} homozygotes (*Met*^{158} 103.8±13.3; *Val*^{158}/ *Val*^{158} 94.1±15.7; p=0.004) (Table 2). *COMT Met*^{158} subjects did not associate with a task requiring mental flexibility on TMT B-A (*Met*^{158} 46.6±51.5; *Val*^{158}/*Val*^{158} 63.8±42.0, p=0.139) (Table 2). *COMT Val*^{158}*Met* polymorphism did not associate with verbal learning and fluency as measured by the CVLT-II Trial 1–5 Standard Score (*Met*^{158} 54.5±11.1; *Val*^{158}/*Val*^{158} 53.7±9.4, p=0.740) (Table 2).

COMT Val^{158}*Met* is associated with nonverbal processing speed after mTBI

To further assess the association between *COMT Val*^{158}*Met* and nonverbal processing speed as measured by the WAIS-PSI composite score, multivariable regression was performed to control for education years, race, and injury severity (Table 3). *COMT Met*^{158} carriers demonstrated higher adjusted mean scores on WAIS-PSI (101.6±2.1) compared to their *Val*^{158}/*Val*^{158} counterparts (93.8±3.0), which corresponds to a mean increase of 7.9 points (95 % CI [1.4 to 14.3], p=0.017) (Fig. 1). Consistent with prior reports [55–57], education years associated with WAIS-PSI (B=1.4, 95 % CI [0.4 to 2.3], p=0.005). Greater injury severity also associated with a decrease in nonverbal processing speed (GCS 15, 101.6±1.9; GCS <15, 93.8±3.0; B=−7.9, 95 % CI [−14.1 to −1.7], p= 0.013). Race did not show a significant association with WAIS-PSI (p=0.539) on multivariable analysis. Further, multivariable subgroup analysis performed in the Caucasian group—the largest group—demonstrated a statistical trend between the *COMT Val*^{158}*Met* polymorphism and performance on WAIS-PSI (B=7.5, 95 % CI [−1.1 to 16.0], p= 0.086). Future studies are needed to confirm this finding in a larger population.

COMT Val^{158}*Met* is not associated with mental flexibility after mTBI

To further assess the association between *COMT Val*^{158}*Met* and mental flexibility as measured by the TMT B-A time, multivariable regression was performed to control for education years, race, and injury severity. Since the TMT B-A has not been intrinsically adjusted for age, we further adjusted for age in the current analysis. *COMT Val*^{158}*Met* did not demonstrate an association with TMT B-A after adjustment (*Met*^{158} 47.7±7.1; *Val*^{158}/*Val*^{158} 58.8±10.2; B=−11.1, 95 % CI [−33.0 to 10.8], p=0.318) (Table 3). Consistent with prior reports [58, 59], both age years (B=1.2, 95 % CI [0.6 to 1.8], p<0.001) and education years (B=−5.2, 95 % CI [−8.4 to −2.0], p=0.002) associated with decreased and increased performance on mental flexibility, respectively. Injury severity did not show a significant association with TMT B-A (GCS 15 47.5±6.5; GCS <15 59.0±10.3; B=11.5,

95 % CI [−9.7 to 32.6], p=0.284). Race did not show a significant association with TMT B-A (p=0.492) on multivariable analysis.

COMT Met^{158} is not associated with verbal learning after mTBI

To further assess the association between *COMT Val*^{158}*Met* and verbal learning as measured by the CVLT-II, multivariable regression was performed to control for education years, race, and injury severity. *COMT Val*^{158}*Met* did not demonstrate an association with CVLT-II after adjustment (*Met*^{158} 50.9±1.6; *Val*^{158}/*Val*^{158} 51.6±2.4; B=−0.7, 95 % CI [−5.8 to 4.3], p=0.771) (Table 3). Consistent with prior reports [60], education years (B=0.6, 95 % CI [−0.1 to 1.4], p=0.098) showed a borderline association with verbal learning. Greater injury severity also associated with a decrease in verbal learning (GCS 15 53.7±1.5; GCS <15 48.7±2.4; B=−5.0, 95 % CI [−9.9 to −0.1], p=0.044). Race showed a borderline significant association with CVLT-II (p=0.068) on multivariable analysis, driven primarily by a difference between the Caucasian subgroup and the heterogeneous “other races” subgroup (B=−5.9 [−11.5 to −0.2], p=0.042).

Discussion

In the present study, we sought to investigate whether the *COMT Val*^{158}*Met* polymorphism is associated with cognitive performance at 6 months following mild closed head injury in an isolated, uncomplicated mTBI population. We found that subjects with the *COMT Met*^{158} allele showed higher performance on a measure of nonverbal processing speed compared to *Val*^{158}/*Val*^{158} homozygotes at 6 months following injury independent of injury severity and race. We also demonstrate that the *COMT Val*^{158}*Met* polymorphism is not associated with a measure of executive control and mental flexibility or a measure of verbal learning after controlling for injury severity and race. We confirm that greater injury severity is associated with poorer nonverbal processing speed and verbal learning. Further, racial stratification was not found to significantly associate with nonverbal processing speed, mental flexibility, or verbal learning after uncomplicated mTBI in the current patient population.

In our current analysis, *COMT Met*^{158} carriers showed an adjusted mean score of 101.6 on the WAIS-PSI, while *Val*^{158}/ *Val*^{158} homozygotes showed 93.8—these scores correspond to the ~55th percentile and the ~34th percentile of nonverbal processing speed performance in the normal population, respectively [44]. We also find that the adjusted mean scores (~50 s) on the CVLT-II correspond to the general mean of the normal population for both *COMT Val*^{158}*Met* groups [50]. Further, the adjusted TMT B-A times for both *COMT* groups fall within the means reported in literature (~40 to ~60) for the normal/uninjured population [49, 61, 62]. Thus, it is worth noting that a subgroup of patients with isolated uncomplicated mTBI demonstrates heightened risk for decreased performance on nonverbal processing, but not verbal learning or executive function at 6 months postinjury, and this subgroup associates with the common SNP *COMT Val*^{158}*Met*.

It is generally accepted that acute physiologic recovery occurs by 6 months post-mTBI on imaging studies [9, 63, 64], and studies report that most cognitive symptoms resolve by within the first 3 months in mTBI [65, 66]. To our knowledge, this is the first study of the association between *COMT Val*^{158}*Met* and cognitive performance at an extended time point

of recovery, such as 6 months following mTBI. Prior reports examining the potential influence of the *COMT Val*^{158}*Met* polymorphism on TBI cognitive outcomes have been conducted during acute and subacute recovery with a mean time of collection within 2 months postinjury and have been predominately limited to patients with moderate and/or severe injuries [17, 18, 67]. For example, in a cohort of 113 TBI rehabilitation patients assessed at a mean of 2 months postinjury,[17] *Val*^{158}/*Val*^{158} homozygotes were found to score lower on a measure of cognitive flexibility—the ability to alter a behavioral response against changing contingencies [68]—and to have a greater number of perseverative errors. In another sample of 32 moderate-to-severe TBI patients with 40 health controls, *COMT Met*^{158} was found to associate with preserved strategic control of attention at 2 months postinjury [67]. In the largest study of *COMT* and moderate-to-severe TBI to date, Willmott et al. did not find an association between *COMT* and measures of cognition at roughly 1 month postinjury [18]. However, this study evaluated cognitive performance at a time point that was not standardized and closer to the time of injury (mean 29 days); the authors suggest that cognitive assessment at 6–12 months postinjury may be more likely to detect subtle group differences as demonstrated in the present report.

There is physiological evidence in support of a potential modulatory role of the *COMT Met*^{158} allele in cognitive performance following TBI. The PFC is a key center for overall executive function, attention, and strategic planning [69–71], in which its rich dopaminergic pathways are more dependent on COMT for regulation and modulation at the synaptic cleft [19–21]. Prior studies have demonstrated that the *COMT Val*^{158}*Met* polymorphism is associated with differences in cognitive performance in the absence of brain injury [23, 72]. Given the absence of measures of baseline preinjury performance in our population or neuropsychiatric data in appropriately uninjured age-matched controls, we cannot conclude whether our results reflect the maintenance of preexisting cognitive differences between genotypes and/or an altered trajectory of recovery or impairment following mTBI.

There are also several additional limitations to the present study. Our data was obtained for a relatively small sample size (n=100) in a predominately Caucasian male population and did not conform to known HapMap Phase III subpopulations; therefore, there is a need for studies of confirmation in similar populations and of validation in larger and more diverse study populations. We also included patients only with isolated mTBI in the absence of intracranial findings on CT and a limited period of diminished consciousness and/or posttraumatic amnesia; thus, the generalizability of our results is limited. We also include no neuroimaging outside of 24 h or magnetic resonance imaging. Therefore, it is possible that a subset of the subjects developed delayed pathology on neuroimaging and would no longer be classified as uncomplicated. We pursued analyses designed to investigate a hypothesized relationship between the *COMT Val*^{158}*Met* polymorphism and cognitive outcome and did not explore the structure-function implications of *COMT* with specific brain pathology or variables important to the trajectory of recovery such as treatment and support. There is also a need to examine gene-gene interaction with other susceptibility loci in the context of mTBI to better elucidate complex interactions and mechanisms through which the *COMT* molecular pathway may influence response and recovery to TBI. Finally, all of our findings must be considered preliminary until they are formally replicated.

Conclusions

The *COMT Val*^{158}*Met* polymorphism (*rs4680*) is associated with nonverbal cognitive performance following uncomplicated mTBI without polytrauma. More specifically, the *COMT Met*^{158} allele is associated with increased performance in nonverbal processing speed, while no associations were seen on mental flexibility or verbal learning. Larger studies in similar populations will be of value to confirm the role of *COMT Val*^{158}*Met* polymorphism in these domains and to explore its effects in other cognitive domains following mTBI. Whether *COMT Val*^{158}/ *Val*^{158} homozygotes would benefit from heightened clinical surveillance and/or pharmacologic and cognitive behavior therapy remains to be determined and may represent an important direction of future studies.

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Appendix

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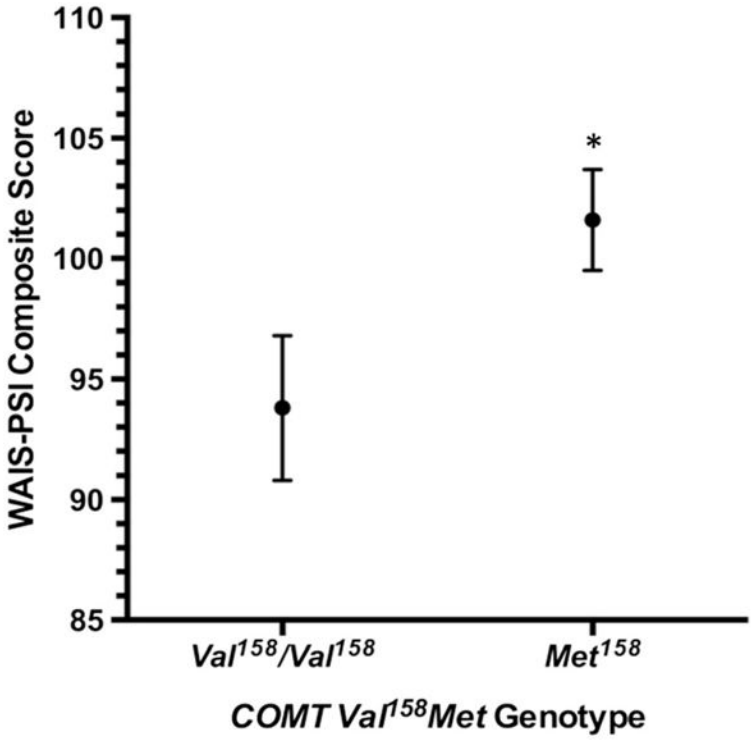

Fig. 1.

COMT Val^{158}*Met* and 6-month WAIS-PSI Composite Score after mild traumatic brain injury. The *COMT Val*^{158}*Met* polymorphism is associated with statistically greater preservation of nonverbal processing speed 6 months following mild traumatic brain injury after adjusting for race, years of education, and injury severity. Means and standard errors on the WAIS-PSI Composite Score are shown for *Met*^{158} and *Val158*/*Val*^{158} genotype groups. *COMT*, Catechol-O-Methyltransferase, *WAIS-PSI* Wechsler Adult Intelligence Scale Fourth Edition—Processing Speed Index. *p<0.05.

Table 1

Demographic and clinical information of included subjects with mild traumatic brain injury

Variable	*COMT Met*^{158} (*N*=76)	*COMT Val*^{158}/*Val*^{158} (*N*=24)	Sig. (*p*)
Age (years)			
Mean±SD	40.5±15.7	42.2±14.1	0.643
Gender			
Male	49 (65 %)	17 (71 %)	0.566
Female	27 (35 %)	7 (29 %)	
Race			
Caucasian	57 (81 %) [a]	13 (19 %) [a]	0.042
African-American/African	7 (50 %) [a]	7 (50 %) [b]	
Other races	12 (75 %) [a]	4 (25 %) [a]	
Education (years)			
Mean±SD	14.6±2.7	13.0±3.1	0.015
Mechanism of injury			
Motor vehicle crash	24 (32 %)	2 (8 %)	0.110
Pedestrian versus auto	17 (22 %)	5 (21 %)	
Fall	23 (30 %)	10 (42 %)	
Assault	9 (12 %)	6 (25 %)	
Struck by/against object	3 (4 %)	1 (4 %)	
Posttraumatic amnesia			
No	30 (40 %)	11 (46 %)	
Yes	42 (55 %)	10 (42 %)	0.310
Unknown	4 (5 %)	3 12 %)	
GCS—field[a]			
<15	21 (36 %)	6 (35 %)	0.982
=15	38 (64 %)	11 (65 %)	
GCS—ED arrival			
<15	19 (25 %)	4 (17 %)	0.579
=15	57 (75 %)	20 (83 %)	
ED disposition			
ED discharge	53 (70 %)	13 (54 %)	0.284
Hospital ward admission	20 (26 %)	10 (42 %)	
ICU admission	3 (4 %)	1 (4 %)	

Race distributions are reported as row percentages. All other distributions reported as column percentages. The race subgroup “other races” was combined due to individual small sample sizes of Asian (*N*=5; *Met*^{158}=4, *Val*^{158}/*Val*^{158}=1), American Indian/Alaskan Native (*N*=1; *Met*^{158}=1), Hawaiian/Pacific Islander (*N*=1; *Met*^{158}=1), and more than one race (*N*=9; *Met*^{158}=6, *Val*^{158}/*Val*^{158}=3)

COMT catechol-O-methyltransferase, *ED* emergency department, *GCS* Glasgow Coma Scale, *ICU* intensive care unit, *SD* standard deviation

[a] Data for GCS—Field was only available for 76 patients

Table 2

Distribution of performance on 6-month cognitive outcome measures following mild traumatic brain injury by *COMT* genotype

Outcome Measure	*Met*^{158} (*N*=76)	*Val*^{158}/*Val*^{158} (*N*=24)	Sig. (*p*)
WAIS-PSI Composite Score[a]	103.8±13.3	94.1±15.7	0.004
TMT Trail B minus A Time[b]	46.6±51.5	63.8±42.0	0.139
CVLT-II Trial 1–5 Standard Score[a]	54.5±11.1	53.7±9.4	0.740

Distributions are reported as mean±standard deviation

COMT catechol-O-methyltransferase, *CVLT-II* California Verbal Learning Test, second edition, *TMT* Trail Making Test, *WAIS-PSI* Wechsler Adult Intelligence Scale, fourth edition, Processing Speed Index

[a] Higher scores suggest improved performance

[b] Lower scores suggest improved performance

Table 3

Multivariable analysis of the *COMT Val*^{158}*Met* polymorphism and 6-month cognitive outcome following mild traumatic brain injury

WAIS-PSI Composite Score[a]	**Mean±SE**	**B [95 % CI]**	**Sig. (*p*)**
COMT Val^{158}*Met*			0.017
Val^{158}/ *Val*^{158}	93.8±3.0	Reference	–
Met^{158}	101.6±2.1	7.9 [1.4, 14.3]	
GCS			0.013
GCS=15	101.6±1.9	Reference	–
GCS <15	93.8±3.0	−7.9 [−14.1, −1.7]	
Race			0.539
Caucasian	96.8±2.1	Reference	–
African-American/African	95.8±3.6	−1.1 [−9.0, 6.9]	0.790
Other	100.5±3.5	3.7 [−3.5, 10.9]	0.312
Education (years)	–	1.4 [0.4, 2.3]	0.005
TMT Trail B minus A Time[b]	Mean±SE	B [95 % CI]	Sig. (*p*)
COMT Val^{158}*Met*			0.318
Val^{158}/ *Val*^{158}	58.8±10.2	Reference	–
Met^{158}	47.7±7.1	−11.1 [−33.0, 10.8]	
GCS			0.284
GCS=15	47.5±6.5	Reference	–
GCS <15	59.0±10.3	11.5 [−9.7, 32.6]	
Race			0.492
Caucasian	59.2±7.1	Reference	–
African-American/African	43.0±12.3	−16.2 [−43.1, 10.7]	0.235
Other	57.4±12.2	−1.8 [−27.0, 23.4]	0.888
Education (years)	–	−5.2 [−8.4, −2.0]	0.002
Age (years)	–	1.2 [0.6, 1.8]	<0.001
CVLT-II Trial 1–5 Standard Score[a]	Mean±SE	B [95 % CI]	Sig. (*p*)
COMT Val^{158}*Met*			0.771
Val^{158}/ *Val*^{158}	51.6±2.4	Reference	–
Met^{158}	50.9±1.6	−0.7 [−5.8, 4.3]	
GCS			0.044
GCS =15	53.7±1.5	Reference	–
GCS <15	48.7±2.4	−5.0 [−9.9, −0.1]	
Race			0.068
Caucasian	54.7±1.6	Reference	–
African-American	50.1±2.8	−4.7 [−10.9, 1.5]	0.139
Other	48.9±2.8	−5.9 [−11.5, −0.2]	0.042
Education (years)	–	0.6 [−0.1, 1.4]	0.098

The WAIS Processing Speed Index (WAIS-PSI) Composite Score and the CVLT-II Trial 1–5 Standard Score are adjusted for education years, race (Caucasian, African-American/African, other races), and GCS (15 vs. less than 15). The TMT Trail B minus ATime is adjusted for age, education years, race, and GCS. Distributions are reported as adjusted mean±standard error. The mean difference (*B*) between *COMT* Met^{158} and *COMT* Val^{158} / Val^{158} and associated 95 % CI is reported for each outcome measure CVLT-II, California Verbal Learning Test, Second Edition; TMT, Trail Making Test; WAIS, Wechsler Adult Intelligence Scale, Fourth Edition.

CI confidence interval, *COMT* catechol-O-methyltransferase, *CVLT-II* California Verbal Learning Test, second edition, *GCS* Glasgow Coma Scale, *TMT* Trail Making Test, *WAIS* Wechsler Adult Intelligence Test

[a] Higher scores suggest improved performance

[b] Lower scores suggest improved performance

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Lab resource

COMT Val^{158}*Met* polymorphism is associated with post-traumatic stress disorder and functional outcome following mild traumatic brain injury

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ABSTRACT

Mild traumatic brain injury (mTBI) results in variable clinical trajectories and outcomes. The source of variability remains unclear, but may involve genetic variations, such as single nucleotide polymorphisms (SNPs). A SNP in catechol-o-methyltransferase (*COMT*) is suggested to influence development of post-traumatic stress disorder (PTSD), but its role in TBI remains unclear. Here, we utilize the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) study to investigate whether the *COMT Val*^{158}*Met* polymorphism is associated with PTSD and global functional outcome as measured by the PTSD Checklist – Civilian Version and Glasgow Outcome Scale Extended (GOSE), respectively. Results in 93 predominately Caucasian subjects with mTBI show that the *COMT Met*^{158} allele is associated with lower incidence of PTSD (univariate odds ratio (OR) of 0.25, 95% CI [0.09–0.69]) and higher GOSE scores (univariate OR 2.87, 95% CI [1.20–6.86]) 6-months following injury. The *COMT Val*^{158}*Met* genotype and PTSD association persists after controlling for race (multivariable OR of 0.29, 95% CI [0.10–0.83]) and pre-existing psychiatric disorders/substance abuse (multivariable OR of 0.32, 95% CI [0.11–0.97]). PTSD emerged as a strong predictor of poorer outcome on GOSE (multivariable OR 0.09, 95% CI [0.03–0.26]), which persists after controlling for age, GCS, and race. When accounting for PTSD in multivariable analysis, the association of *COMT* genotype and GOSE did not remain significant (multivariable OR 1.73, 95% CI [0.69–4.35]). Whether *COMT* genotype indirectly influences global functional outcome through PTSD remains to be determined and larger studies in more diverse populations are needed to confirm these findings.

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1. Introduction

Traumatic brain injury (TBI) is a common and often debilitating injury in modern societies. In the United States alone, at least 2.5 million people suffer TBIs annually which accounts for 52,000 deaths, 275,000 inpatient hospitalizations, and 1,365,000 Emergency Room visits [1]. Approximately 70–90% of all TBI is characterized as 'mild TBI' (mTBI) defined by a Glasgow Coma Scale score of 13–15. The vast majority of patients make a complete recovery following mTBI in the ensuing weeks to months [2,3]. However, a small but significant number of patients suffer from persistent neurologic, cognitive and/or neuropsychiatric sequelae – including headache, dizziness, fatigue, memory impairment, slowed processing speed, depression and post-traumatic stress disorder (PTSD), among others [4]. In many instances, individuals enduring similar mechanisms and magnitude of brain injury follow different clinical trajectories, and there are limited metrics to identify and/or sub-stratify those at greatest risk for persistent post-traumatic impairment [5].

The Human Genome Project has allowed the identification of single nucleotide polymorphisms (SNPs) – single nucleotide substitutions which alter amino acid sequence and protein function and/or levels of protein expression. Several SNPs are good candidates for allelic association studies aimed at explaining the divergence in outcome or in the prevalence of cognitive, behavioral and neuropsychiatric symptoms following TBI [6–8]. Catechol-*O*-methyltransferase (*COMT*; encoded by the gene *COMT* on chromosome 22q11.2) represents one such candidate gene. *COMT* enzymatically inactivates the catecholamine neurotransmitters, i.e., norepinephrine and dopamine, through 3-*O*-methylation of the benzene ring [9]. A common coding SNP, $Val^{158}Met$ (*rs4680*), results in a substitution of valine (G; Val^{158}) for methionine (A; Met^{158}) at codon 158. The Met^{158} substitution reduces *COMT* enzymatic activity [10,11]. In areas with limited reuptake transporters, i.e., the prefrontal cortex, *COMT*-mediated inactivation is the principal mechanism of inactivation of dopaminergic signal transmission, and the Met^{158} variant is associated with higher catecholamine bioavailability [12–14].

Numerous studies have investigated the effects of the *COMT* $Val^{158}Met$ polymorphism on human behavior and/or brain function [9,15,16]. In general, the Met^{158} allele confers a performance advantage in cognitive tasks – including measures of memory and attention – attributed to the higher catecholamine bioavailability in the prefrontal cortex [9,15–17]. The Val^{158} allele, on the other hand, may confer advantage in aversive stimulus processing [18]. Consistent with this principle, the Met^{158} allele has been associated with a number of anxiety disorders – including generalized anxiety disorder, panic disorder, obsessive compulsive disorder, and PTSD in some, but not all study populations [19–23]. In TBI, prior studies have shown that Val^{158}/Val^{158} homozygosity may be associated with greater impairment in certain cognitive domains, e.g., perseveration, but not others [24–27]. However, whether the *COMT* $Val^{158}Met$ polymorphism influences psychiatric health following brain injury – such as TBI – has yet to be studied.

PTSD is a relatively common and often debilitating neuropsychiatric sequela of TBI with published rates ranging from of 17% to 33% of patients [28–32]. The Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) classifies PTSD as an anxiety disorder presenting with three concurrent symptom clusters after exposure to a traumatic event: persistent re-experiencing of the traumatic event, persistent avoidance of stimuli associated with the traumatic event, and persistent symptoms of increased arousal. The event can involve actual or perceived serious injury, a threat to one's physical integrity or the integrity of another individual, or an unexpected death or serious harm to a close family member or friend. The symptoms must be present for more than one month and cause clinically significant distress or impairment in social, occupational, or other important areas of functioning [33]. Although PTSD may occur with any severity of TBI, the highest incidence occurs in individuals with mTBI [31,34–36]. Furthermore, symptoms of PTSD in the context of a history of mTBI is often associated with poorer outcome [37].

In this study we utilize the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) dataset, a comprehensive database of demographic history, biomarkers, neuroimaging, and neuropsychiatric and neurocognitive outcomes [38], to investigate whether the *COMT* $Val^{158}Met$ polymorphism is associated with the development of symptoms meeting DSM-IV criteria of PTSD and global functional outcome following isolated and uncomplicated mild closed head injury. On the basis of the literature of anxiety disorders, we hypothesize that subjects with the Met^{158} allele will more frequently develop symptoms associated with PTSD and have poorer 6-month global functional outcome following mTBI.

2. Materials and methods

2.1. Study design

The TRACK-TBI Pilot Study is a multicenter prospective observational study conducted at three Level 1 Trauma Centers in the United States – San Francisco General Hospital, University of Pittsburgh Medical Center and University Medical Center Brackenridge (UMCB) in Austin, Texas [38]. Institutional review board approval was obtained at all participating sites. Informed consent was obtained for all subjects prior to enrollment in the study. For patients unable to provide consent due to their injury, consent was obtained from their legally authorized representative (LAR). Patients were then re-consented, if cognitively able at later inpatient and/or outpatient follow-up assessments for continued participation in the study.

2.2. Patient selection

Inclusion criteria for the pilot study were patients presenting to a Level I trauma center with external force trauma to the head and clinically indicated head computed tomography (CT) scan within 24 h of injury. TRACK-TBI Pilot patients age $\geqslant$16 completed outcome measures. Exclusion criteria were pregnancy, comorbid life-threatening disease, incarceration, serious psychiatric and neurologic disorders that would interfere with outcome assessment, and non-English speakers due to limitations in participation with outcome assessment. For the present study, our goal was to study the effects of *COMT* $Val^{158}Met$ in isolated and uncomplicated mTBI. Therefore, our analysis was restricted to a subset of patients with a GCS $\geqslant$13, loss of consciousness (LOC) <30 min, post-traumatic amnesia <24 h, no skull fracture or acute intracranial pathology – defined as the absence of intraparenchymal contusions or hemorrhage, axonal injury, ventricular hemorrhage, epidural hematoma, acute subdural hematoma or traumatic subarachnoid hemorrhage – on non-contrasted head CT, and no polytrauma as defined by an Abbreviated Injury Scale (AIS) score >1 in any extracranial body region. To avoid potential confounding with measures of PTSD, patients who reported pre-injury PTSD or schizophrenia – variables known to associate with *COMT* – were excluded from the present study [39,40]. Patients with previous cerebrovascular accidents, brain tumor, and baseline developmental delay were also excluded.

2.3. Biospecimen collection and genotyping

Specimen acquisition was performed as previously described [38]. In brief, blood samples for DNA genotyping analysis were collected via peripheral venipuncture or existing peripheral venous indwelling catheters within 24 h of injury. Samples were collected in BD Vacutainer K_2-EDTA vacutainer tubes, and subsequently aliquoted and frozen in cryotubes at −80 °C within 1 h of collection in accordance with recommendations from the NIH-CDE Biomarkers Working Group [41]. DNA was extracted from isolated leukocytes using the Wizard® Genomic DNA Purification Kit as described by the manufacturer (Promega, Madison, WI). *COMT* Val158Met polymorphism (rs4680) was genotyped utilizing TaqMan®SNP Genotyping Assay as described by the manufacturer (Applied Biosystems, Carlsbad, CA). For the purposes of evaluating a potential protective benefit of the Met^{158} allele, Met^{158}/Met^{158} and Met^{158}/Val^{158} were combined as a single group as previously described for *COMT* [42–45], and other genetic polymorphisms in TBI [46–48]. Therefore, for data recording and all figures this group is referred to as Met^{158}.

2.4. Neuropsychiatric assessment and outcome parameters

All participants underwent a neuropsychiatric and outcome assessment at 6 months following TBI, including the PTSD Checklist – Civilian Version (PCL-C) and the Glasgow Outcome Scale Extended (GOSE). To evaluate for the presence of PTSD, the PCL-C was utilized as previously described [49–51]. The PCL-C is a standardized self-report rating scale of 17 PTSD symptoms that can be mapped to the three required criteria for PTSD, as outlined in the DSM-IV. Respondents are asked to rate on a 5-point scale (1-"not at all" to 5-"extremely") how much they have been bothered by each symptom in the past month. Subjects endorsing a score of $\geqslant 3$ in one or more symptoms in "Re-experiencing", three or more symptoms in "Avoidance", and two or more symptoms in the "Hypervigilance" categories on the PCL-C were coded as "Yes PTSD" during analysis in accordance to the DSM-IV clinical screening criteria for PTSD.

The GOSE was utilized to assess global functional outcome following TBI as previously described [52]. The GOSE provides an overall measure of disability based on information obtained through a structured interview focused on cognition, independence, employability, and social/community participation. Individuals are described by one of the eight ordinal outcome categories: 1-Dead, 2-Vegetative State, 3-Lower Severe Disability, 4-Upper Severe Disability, 5-Lower Moderate Disability, 6-Upper Moderate Disability, 7-Lower Good Recovery, and 8-Upper Good Recovery.

2.5. Statistical analysis

Group differences in baseline descriptors across *COMT* Met^{158} carriers versus Val^{158}/Val^{158} homozygotes were assessed by Pearson's chi-squared test (χ^2) for categorical variables, and analysis of variance (ANOVA) for continuous variables. Fisher's Exact Test was used to assess differences in categorical variables with cell counts $\leqslant 5$. Predictors examined in addition to *COMT* genotype were selected on the basis of prior published reports, and limited to variables that were previously associated with the response variable and relevant within our study population of isolated, uncomplicated mTBI to help control for potential confounding in multivariable analyses. Given the constraints of our exclusion criteria, remaining variables known to associate with PTSD include the presence of a self-reported pre-existing psychiatric disorder (defined by the major categories of diagnosed depression, anxiety, sleep disorder, and bipolar disorder) and history of substance abuse [2,53–55]. For GOSE, age (per-year increase) and GCS (15 vs. 13–14) were selected as consistent predictors cited in literature [56]. Binary logistic regression was performed with PTSD as the response, and *COMT* genotype, presence of pre-existing psychiatric disorder, and illicit drug use history as predictors. Ordinal logistic regression was performed with GOSE as the response, and *COMT* genotype, age, and GCS as predictors. All multivariable regression models conformed to tests for goodness-of-fit. The Nagelkerke pseudo-R-square (NR^2) used to estimate the variance explained by the model. Race effects were independently assessed in the presence of *COMT* genotype for each response. Significance was assessed at $\alpha = 0.05$. All analyses were performed using Statistical Package for the Social Sciences (SPSS) v.21 (IBM Corporation, Chicago, IL).

3. Results

3.1. Demographic and clinical descriptors

In total, the present study included 93 subjects (Table 1). Overall, the mean age of included subjects was 40 years old, and the majority of subjects were male (60%). Subjects were predominately Caucasian (70%). Subjects also self-identified as African American (14%), Asian (7%), mixed race (7%), American Indian/Native Alaskan (2%) or Hawaiian/Pacific Islander (2%). With respect to psychiatric health, 39% of subjects had self-reported presence of one or more psychiatric conditions – including depression, anxiety, sleep disorder, or bipolar disorder – and 24% percent of subjects reported a history of substance abuse. Subjects had a multitude of different mechanisms of injury including motor vehicle or motorcycle collision, bicycle accident, pedestrian versus automobile, assault and

Table 1
Demographic and clinical information of included subjects with mild traumatic brain injury.

Variable	Met^{158} ($N = 70$)	Val^{158}/Val^{158} ($N = 23$)	Significance (p)
Age (y)			
Mean ± SD	40 ± 17	42 ± 14	0.682
Gender			
Male	42 (60%)	14 (61%)	0.941
Female	28 (40%)	9 (39%)	
Race			
Caucasian	52 (80%)	13 (20%)	0.032
African-American/African	6 (46%)	7 (54%)	
Other races	12 (80%)	3 (20%)	
Pre-existing psychiatric disorder			
No	47 (67%)	10 (44%)	0.043
Yes	23 (33%)	13 (56%)	
Substance abuse			
No	56 (80%)	15 (65%)	0.148
Yes	14 (20%)	8 (35%)	
Mechanism of injury			
Motor vehicle crash	22 (31%)	2 (9%)	0.140
Cyclist/pedestrian hit	15 (21%)	6 (26%)	
Fall	21 (30%)	8 (35%)	
Assault	8 (11%)	6 (26%)	
Struck by/against object	4 (6%)	1 (4%)	
ED arrival GCS			
13	1 (1%)	0 (0%)	0.817
14	12 (17%)	5 (22%)	
15	57 (81%)	18 (78%)	

Race distributions are reported as row percentages. All other distributions reported as column percentages. The race subgroup "Other races" was combined due to individual small sample sizes of Asian ($N = 6$; $Met^{158} = 5$, $Val^{158}/Val^{158} = 1$), American Indian/Alaskan Native ($N = 1$; $Met^{158} = 1$), Hawaiian/Pacific Islander ($N = 2$; $Met^{158} = 1$, $Val^{158}/Val^{158} = 1$), and more than one race ($N = 6$; $Met^{158} = 5$, $Val^{158}/Val^{158} = 1$).

struck by or against an object. *COMT* genotype distribution was 29% Met^{158}/Met^{158} ($n = 27$), 46% Met^{158}/Val^{158} ($n = 43$) and 25% Val^{158}/Val^{158} ($n = 23$). *COMT* allelic frequencies ($A = 0.53$, $G = 0.47$) were not found to deviate significantly from Hardy–Weinberg equilibrium ($X^2 = 0.5$, $p = 0.778$). A higher prevalence of African-Americans ($p = 0.032$) and preexisting psychiatric disorder ($p = 0.043$) were noted in the Val^{158}/Val^{158} homozygotes. No other significant differences were observed in the distribution of each demographic and clinical descriptor across *COMT* Met^{158} and Val^{158}/Val^{158} genotypes (Table 1).

3.2. COMT is associated with PTSD independent of pre-existing psychiatric disease, substance abuse and race

We first tested our hypothesis that *COMT* Met^{158} is associated with higher incidence of PTSD following mTBI. In total, 28 of 96 subjects (29%) qualified for PTSD on screening criteria. Sixteen of 73 (22%) of Met^{158} carriers and 12 of 23 (52%) of Val^{158}/Val^{158} homozygotes met qualifying screening criteria for PTSD, respectively ($X^2 = 7.75$, $p = 0.005$) (Fig. 1). *COMT* Met^{158} had a univariate odds ratio (OR) of 0.25 (95% CI [0.09–0.69], NR^2 11.0%) for the presence of PTSD (Table 2). Therefore, contrary to our initial hypothesis, *COMT* Met^{158} was associated with lower incidence of PTSD.

Given that *COMT* genotype unevenly distributed across racial subgroups, we next utilized multivariable regression for PTSD to control for the potential confounding influence of race. In the studied population, univariate analysis failed to show a statistically significant relationship between race and PTSD following mTBI ($p = 0.092$). When only the two largest racial groups were compared populations were compared (Caucasian and African American), African American race emerged as a predictor for PTSD with a univariable OR of 3.89 (95% CI [1.13–13.35]). However, only COMT genotype, but not African American racial background, remained a statistically significant predictor of PTSD when included in a multivariable model as evidenced by a multivariable OR of 0.29 (95% CI [0.10–0.83]) and 2.76 (95% CI [0.75–10.22]) for the *COMT* Met^{158} allele and African American race, respectively. Collectively, these data preliminarily suggest that the association of *COMT* and PTSD is not influenced by race, but larger future studies in more diverse populations are needed to confirm and/or refute these findings.

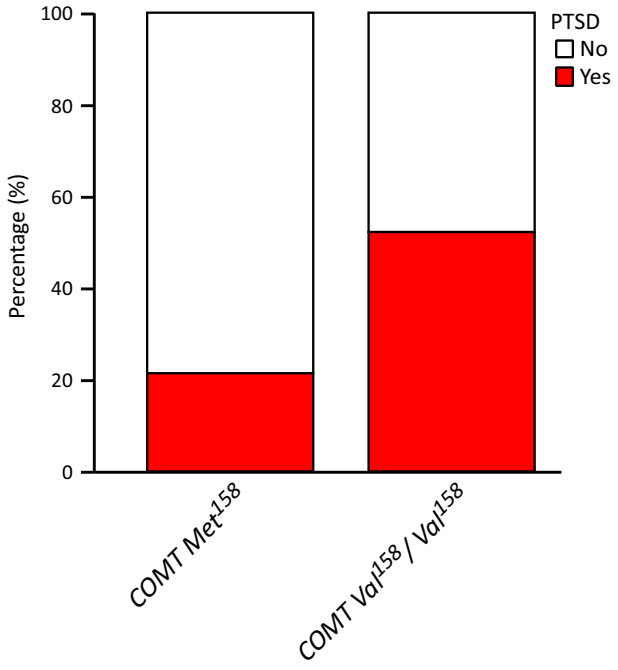

Fig. 1. The *COMT* $Val^{158}Met$ polymorphism is associated with lower prevalence of qualifying for screening criteria for post-traumatic stress disorder (PTSD) at 6-months following mild traumatic brain injury. White, did not meet PTSD qualification on screening criteria; red, met PTSD qualification on screening criteria. *COMT* = Catechol-O-Methyltransferase. red, met PTSD qualification on screening criteria.

We next sought to determine whether controlling for established risk factors for PTSD, e.g., pre-existing psychiatric disease and substance abuse [2,53–55], would influence the observed association between *COMT* genotype and PTSD. Univariate analysis confirmed that pre-existing psychiatric disorder (OR 6.85, 95% CI [2.54–18.49], NR^2 22.6%) and substance abuse (OR 3.44, 95% CI [1.26–9.38], NR^2 8.6%) was associated with greater univariate odds of PTSD (Table 2). We also confirmed that there was no effect of interaction between *COMT* and preexisting psychiatric disorder on PTSD ($p = 0.195$). We then performed multivariable regression with PTSD as the response and COMT, preexisting psychiatric disorder, and substance abuse as predictors. In the multivariable model *COMT* Met^{158} remained a significant predictor of decreased odds of PTSD (OR 0.32, 95% CI [0.11–0.97]) after adjusting for pre-existing psychiatric disorder and illicit drug use. In the model, pre-existing psychiatric disorder associated with greater odds of PTSD (OR 5.17, 95% CI [1.80–14.89]) while drug use did not significantly associate with PTSD (Table 2). This model was statistically significant ($p = 8.1 \times 10^{-5}$) and explained 29.5% of the variability in PTSD – values higher than *COMT* Met^{158} or any pre-existing risk factor alone.

3.3. Functional outcome is associated with COMT and inversely related to PTSD

We next investigated whether an association exists between *COMT* Met^{158} carriers or Val^{158}/Val^{158} homozygotes and functional outcome following mTBI. For the 70 *COMT* Met^{158} carriers, 6%, 17%, 37%, and 40% were found to have GOSE scores of 5, 6, 7, 8, respectively. In comparison, 35%, 9%, 30% and 26% of the 23 *COMT* Val^{158}/Val^{158} homozygotes were found to have GOSE scores of 5, 6, 7, 8, respectively, which differed from Met^{158} carriers ($p = 0.008$) (Fig. 2). Univariate ordinal logistic regression showed that the presence of *COMT* Met^{158} allele was associated with an OR of 2.87 for higher GOSE scores (95% CI [1.20–6.86]) and explained 5.9% of the variance. Race was not a significant univariate predictor of GOSE ($p = 0.158$). After correcting for age (per-year increase: univariate OR 0.99, 95% CI [0.97–1.02]; multivariable OR 0.99, 95% CI [0.97–1.02]), and no GCS deficit (GCS 15: univariate OR 2.55 95% CI [1.00–6.57]; multivariable OR 2.68, 95% CI [1.03–6.94]), *COMT* Met^{158} remained a significant predictor of higher functional outcome on GOSE (OR 2.96, 95% CI [1.23–7.13], NR^2 6.2%).

Given the lower incidence of PTSD with *COMT* Met^{158}, we sought to determine whether the observed association between GOSE and *COMT* may in part be explained by the differences in the incidence of post-TBI PTSD between *COMT* Met^{158} carriers or Val^{158}/Val^{158} homozygotes. Overall, low GOSE scores were associated with a higher incidence of PTSD (Fig. 3A–C) Among all subjects and Met^{158} carriers a statistically significant increase in PTSD was observed with decreasing GOSE groups as reflected by p-values of 5.32×10^{-7} and 2.27×10^{-5}, respectively (Fig. 3A and B). Overall a similar trend was observed in Val^{158}/Val^{158} homozygotes (Fig. 3C), but this failed to reach statistical significance ($p = 0.087$). Collectively, this suggests that PTSD may influence functional outcome as previously described for other outcome metrics [57].

To offer further support to this hypothesized relationship, we verified that PTSD is a univariate predictor of lower GOSE by ordinal logistic regression (OR 0.08, 95% CI [0.03–0.21]), and explains 30.6% of the variance in GOSE. We next performed a multivariable

Table 2
Univariate and multivariable statistical analysis of the association between the *COMT Val^{158}Met* polymorphism and a history of preexisting psychiatric disease or substance abuse with post-traumatic stress disorder (PTSD) at 6-months post-injury.

Predictor	Odds ratio (OR) [95% CI]	Predictor sig. (p)	Nagelkerke pseudo-R^2	Model sig. (p)
Univariate analysis				
COMT *Met^{158}*	0.25 [0.09–0.69]	0.006	11.0%	–
Preexisting psychiatric disorder	6.85 [2.54–18.49]	6.3×10^{-5}	22.6%	–
Substance abuse	3.44 [1.26–9.38]	0.016	8.6%	–
Multivariable analysis				
COMT *Met^{158}*	0.32 [0.11–0.97]	0.044	29.5%	8.1×10^{-5}
Preexisting psychiatric disorder	5.17 [1.80–14.89]	0.002	–	–
Substance abuse	1.88 [0.60–5.88]	0.281		

OR >1 represents greater odds of having six-month PTSD. CI = Confidence Interval; *COMT* = Catechol-O-Methyltransferase; OR = Odds Ratio; PTSD = post-traumatic stress disorder.

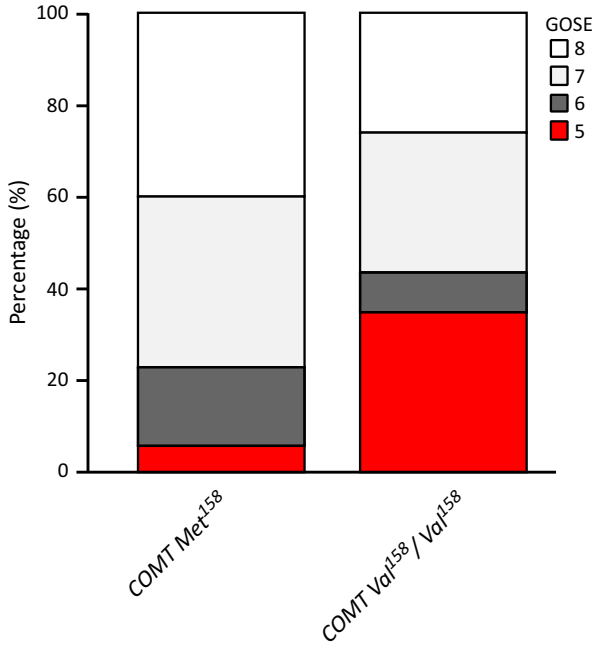

Fig. 2. The *COMT Val^{158}Met* polymorphism is associated with greater global functional outcome as measured by the Glasgow Outcome Scale Extended (GOSE) at 6-months following mild traumatic brain injury. White, GOSE score of 8; light gray, GOSE score of 7; dark gray, GOSE score of 5; red, GOSE score of 5. *COMT* = Catechol-O-Methyltransferase.

ordinal logistic regression with *COMT Met^{158}* and PTSD as predictors, and GOSE as the dependent variable, adjusting for age. The model was statistically significant ($p = 9.06 \times 10^{-7}$) and explained 32.8% of the variability in GOSE. Analysis confirmed that PTSD was associated with greater odds of lower GOSE score as evidenced by multivariable OR of 0.09 (95% CI [0.03–0.26]), but the association of *COMT* with global functional outcome was no longer statistically significant (multivariable OR 1.73, 95% CI [0.69–4.35]) (Table 3). These analyses suggest that PTSD is inversely associated with GOSE and may indirectly contribute to the association of COMT and GOSE following mTBI. However, the directionality of this relationship could not be conferred by the present analysis and future studies are needed to more clearly delineate the influences of PTSD on functional outcome.

4. Discussion

In the present study, we sought to investigate whether the *COMT Val^{158}Met* polymorphism is associated with PTSD and functional outcome following mild closed head injury in a predominately Caucasian population. We found that subjects with the *COMT Met^{158}* allele have lower rates of PTSD and better functional outcomes when compared to *Val^{158}/Val^{158}* homozygotes at 6-months following injury. Much of the effect on functional outcome may be related to differences in PTSD between *COMT* genotypes. How PTSD relates to outcome measures, such as GOSE, remains unclear and future studies addressing this issue are needed.

Prior reports examining the potential influence of the *COMT Val^{158}Met* polymorphism on long-term outcomes following TBI

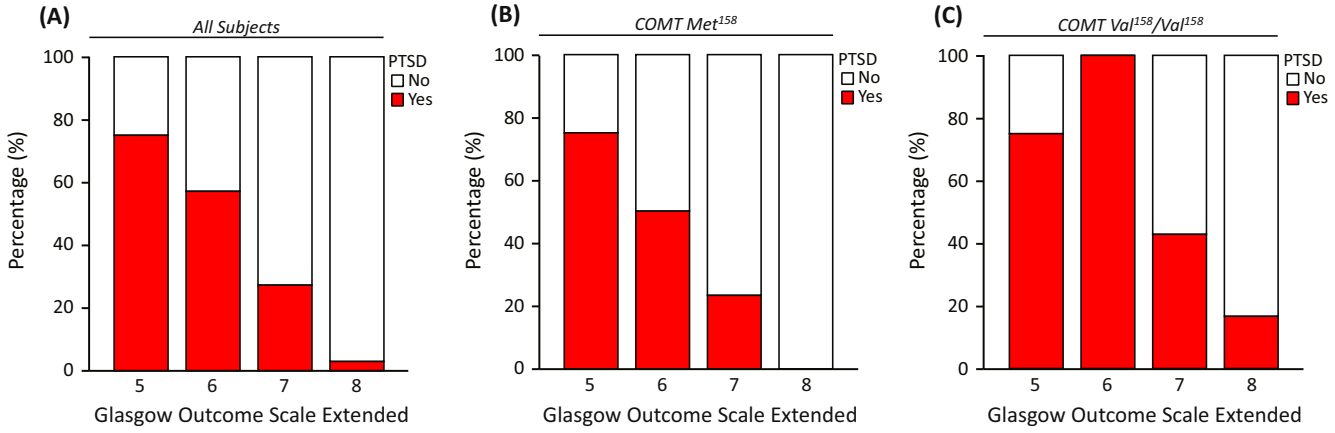

Fig. 3. Global functional outcome is negatively associated with the presence of concomitant post-traumatic stress disorder at 6-months post-injury. (A–C) Graphs depicting proportion of individuals not meeting (white) or meeting (red) post-traumatic stress disorder (PTSD) screening criteria in all subjects (A), subjects with the *COMT Met^{158}* allele (B), and subjects with *COMT Val^{158}/Val^{158}* homozygosity (C). *COMT* = Catechol-O-Methyltransferase.

Table 3
Univariate and multivariable statistical analysis of the association between the *COMT Val^{158}Met* polymorphism and post-traumatic stress disorder (PTSD) with global functional outcome (GOSE) at six months post-injury.

Predictor	Odds ratio (OR) [95% CI]	Predictor sig.(p)	Nagelkerke pseudo-R^2	Model sig. (p)
Univariate analysis				
COMT *Met^{158}*	2.87 (1.20–6.86)	0.018	5.9%	–
PTSD	0.08 [0.03–0.21]	3.62×10^{-7}	30.6%	–
Age (y)	0.99 [0.97–1.02]	0.499	0.5%	–
No GCS deficit	2.55 [1.00–6.57]	0.051	3.9%	–
Multivariable analysis				
COMT *Met^{158}*	1.73 [0.69–4.35]	0.243	32.8%	9.06×10^{-7}
PTSD	0.09 [0.03–0.26]	5.0×10^{-6}	–	–
Age (y)	1.00 [0.98–1.03]	0.723		
No GCS deficit	1.86 [0.69–5.01]	0.218		

OR >1 represents greater odds of higher six-month functional outcome score on GOSE. *COMT* = Catechol-O-Methyltransferase; CI = Confidence Interval; GOSE = Glasgow Outcome Scale Extended; OR = Odds Ratio; PTSD = post-traumatic stress disorder.

have been predominately restricted to measures of cognition in patients with predominantly moderate and severe TBI, with varying results [24–27]. Consistent with a potential deleterious role of *COMT Val^{158}/Val^{158}* in TBI, homozygotes have been shown to have a greater number of perseverative errors following TBI [24,25]. More recently, no relationship between the *COMT Met^{158}-Val* genotype and cognitive performance was found; however, this study did not include measures of perseveration. The authors also failed to find an association between *COMT Val^{158}Met* polymorphism and functional outcome as measured by the GOSE at 12 and 24 months post-injury [27]. The source of this discrepancy with the present report is unclear. The incidence of PTSD is greatest following mTBI [31,34–36], and greater impairment of cognition or prolonged amnesia with more severe TBI may protect against development of subsequent PTSD [58]. Therefore, subjects with more severe injury as studied by Willmott et al., 2014, may not show similar outcome associations in the absence of PTSD. Whether this reflects differences in severity of injury in its entirety and/or trial design – namely interval of follow-up (6-months vs. 12- and 24-months) – or a combination thereof remains to be determined.

To the best of our knowledge, no prior reports have been published investigating the relationship between the *COMT Val^{158}Met* polymorphism and development of PTSD following head trauma. The rate of PTSD of 26.5% in this study is within the published range for mTBI [28–32]. The role of *COMT* in the development of PTSD following other forms of emotional and/or physical trauma remains unclear [21–23,59]. For example, in survivors of the Rwandan genocide, *COMT Met^{158}/Met^{158}* homozygotes demonstrated higher risk for PTSD independent of their traumatic load [21]. The *COMT Met^{158}* allele has also been associated with PTSD following urban violence [22]. However, these studies were conducted in an African and Brazilian population, respectively. It has recently been demonstrated that *COMT Val^{158}Met* polymorphism exerts differential effects on risk of PTSD in children or different ethnic groups [20]; this suggests that different ethnic backgrounds and presumably heterogeneity of genetic modifiers therein modulates the effects of the *COMT Val^{158}Met* polymorphism on genetic propensity for PTSD. Consistent with this principle, a study of predominately Caucasian veterans following deployment to Iraq failed to find an association between *Met^{158}/Met^{158}* and more prevalent PTSD, but did demonstrate that *Met^{158}/Val^{158}* heterozygotes developed fewer PTSD symptoms than either homozygous group [59]. Gene functions and associated phenotypic manifestations are modified through a complex interplay with environmental stimuli [6]. Therefore, whether the predominately Caucasian population, the nature and severity of traumatic event and/or combination thereof contribute to the potentially protective effect of the *COMT Met^{158}* allele following mTBI remains to be seen. We show that the association between *COMT* and PTSD following mTBI persists despite controlling for race. However, stratification of our population into racial groups showed a trend towards lower PTSD with the presence of the *COMT Met^{158}* allele in all racial groups, but failed to reach statistical significance which was in part likely the result of the small sample size of each racial group. Larger studies are therefore needed to fully delineate the potential modifying influence of ethnicity on behavioral and psychiatric associations with *COMT Val^{158}Met* in the setting of head trauma.

The mechanism(s) through which *COMT* influences propensity to develop PTSD also remain unclear. In support of a potentially protective role of the *COMT Met^{158}* allele, a recent study has shown that *COMT Val^{158}/Val^{158}* homozygotes are associated with heightened reacquisition of fear from presumed alterations in reconsolidation of fearful memories [42]. Conversely, the *COMT Met^{158}* allele has been also been associated with impaired fear extinction in some, but not all studies [60,61], and may therefore increase propensity for PTSD development in other contexts. However, a detailed review of the hypothesized mechanisms is beyond the scope of the present study.

Although we designed our study with restrictive inclusion criteria, it is not without limitations. Our data was obtained for a relatively small sample size ($n = 93$) in a predominately Caucasian male population and did not conform to known HapMap Phase III subpopulations; therefore, the need for studies in larger and more diverse study populations cannot be overstated. We also included patients only with isolated mTBI in the absence of intracranial findings on CT and a limited period of diminished consciousness and/or post-traumatic amnesia; thus, the generalizability of our results is limited. We pursued analyses designed to investigate a hypothesized relationship between the *COMT Val^{158}Met* polymorphism and PTSD and did not explore the structure–function implications of *COMT* with specific brain pathology or variables important to the trajectory of recovery such as treatment and support. There is also a need to examine gene-gene interaction with other susceptibility loci in the context of mTBI to better elucidate complex interactions and mechanisms through which the *COMT* molecular pathway may influence response and recovery to TBI.

4.1. Conclusions

The *COMT Val^{158}Met* polymorphism (rs4680) is associated with incidence of PTSD and functional outcome following isolated, uncomplicated mTBI. The *COMT Met^{158}* allele is associated with lower incidence of PTSD and improved functional outcome, and may exert a protective effect. However, larger studies in more diverse populations are needed to confirm the role of *COMT Met^{158}Val* in psychological health following mTBI. Whether

COMT Val^{158}/Val^{158} homozygotes would benefit from heightened clinical surveillance and/or pharmacologic and behavior therapy targeted towards symptomatic manifestations of PTSD remain to be determined and should be the subject of future studies.

Conflict of interest

No competing financial interests exist.

Disclosure statement

The authors have no competing interests to disclose.

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ORIGINAL ARTICLE

Association of a common genetic variant within ANKK1 with six-month cognitive performance after traumatic brain injury

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Abstract Genetic association analyses suggest that certain common single nucleotide polymorphisms (SNPs) may adversely impact recovery from traumatic brain injury (TBI). Delineating their causal relationship may aid in development of novel interventions and in identifying patients likely to respond to targeted therapies. We examined the influence of the (C/T) SNP rs1800497 of ANKK1 on post-TBI outcome using data from two prospective multicenter studies: the

John K. Yue and Angela M. Pronger contributed equally to the manuscript

The COBRIT Investigators and the TRACK-TBI Investigators are listed in the Appendix in alphabetical order by last name

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Citicoline Brain Injury Treatment (COBRIT) trial and Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot). We included patients with ANKK1 genotyping results and cognitive outcomes at six months post-TBI (n=492: COBRIT n=272, TRACK-TBI Pilot n=220). Using the California Verbal Learning Test Second Edition (CVLT-II) Trial 1-5 Standard Score, we found a dose-dependent effect for the T allele, with T/T homozygotes scoring lowest on the CVLT-II Trial 1-5 Standard Score (T/T 45.1, C/T 51.1, C/C 52.1, ANOVA, p=0.008). Post hoc testing with multiple comparison-correction indicated that T/T patients performed significantly worse than C/T and C/C patients. Similar effects were observed in a test of non-verbal processing (Wechsler Adult Intelligence Scale, Processing Speed Index). Our findings extend those of previous studies reporting a negative relationship of the ANKK1 T allele with cognitive performance after TBI. In this study, we demonstrate the value of pooling shared clinical, biomarker, and outcome variables from two large datasets applying the NIH TBI Common Data Elements. The results have implications for future multicenter investigations to further elucidate the role of ANKK1 in post-TBI outcome.

Keywords Traumatic brain injury · Genetic factors · Cognition · Outcome measures · Human studies

Introduction

Traumatic brain injury (TBI) is a complicated injury in a complex organ. Each year in the USA, at least 2.5 million people suffer TBIs. This includes 52,000 deaths, 275,000 hospitalizations, and 1.365 million treated and released from an emergency department (ED) [1]. TBI is a contributing factor to 30 % of all injury-related deaths in the USA [1]. An estimated 3.2 to 5.3 million persons currently live with long-term physical, cognitive, and neuropsychiatric disabilities attributable to TBI [2]. Heterogeneity of the primary injury is complicated by a host of patient-specific factors that together determine clinical outcome [3]. Understanding how physiological factors influence patient outcome provides an avenue for identifying methods of clinical intervention, as well as the patients most likely to benefit. The advent of the Human Genome Project and genetic association analyses has allowed the identification of several polymorphic alleles of candidate genes that may signal disparate outcomes following TBI. However, examination of large numbers of genes results in high chance of type 1 error, underscoring the need for repeat studies of larger samples and high statistical power [4].

Cognitive deficits are among the leading sources of morbidity in TBI patients, and the underlying mechanisms are poorly understood. Patients presenting with similar injuries exhibit disparate patterns of cognitive impairment. The source of this variability is presently unknown but may involve genetic modulation as well as subtle morphometric differences in injury characteristics, highlighting the importance of investigating genetic differences that modulate cognitive function [5]. Previous studies have examined genes that modulate the dopaminergic pathway, which is critical to attention, memory, and executive function. As a result, the dopaminergic system is frequently targeted, through pharmacologic manipulation, to ameliorate chronic deficits in these areas following TBI [6].

Ankyrin repeat and kinase domain-containing 1 (ANKK1) is a candidate gene involved in dopamine transmission [7, 8]. In human adults, ANKK1 mRNA and protein is expressed in the central nervous system (CNS), exclusively in astrocytes [9]. The ANKK1 protein, also known as SgK288, shares structural homology with a family of serine/threonine receptor-interacting protein kinases (RIPKs) potentially responsible for signal transduction and cellular response modulation of dopaminergic reward processes [10].

A common single nucleotide polymorphism (SNP) in the ANKK1 gene may impact outcome after TBI [11, 12]. The C/T SNP rs1800497, also known as Taq1A, is located on chromosome band 11q23.1 in exon 8 of ANKK1 and causes a p. Glu713Lys amino acid change in the C-terminal ankyrin repeat domain, which is involved in protein-protein interaction [10]. Rs1800497 is located 10 kB downstream of the DRD2 gene. While unlikely to directly control DRD2 expression, it may be located within a regulatory region for a functional SNP in the DRD2 gene [10]. Positron emission tomography (PET) studies have shown that rs1800497 affects dopamine binding in the striatum in healthy volunteers [13]. Presence of a single T allele is associated with a 30–40 % reduction of dopamine D2 receptor (DRD2) density in the ventral striatum compared to homozygotes with C alleles, suggesting that T allele carriers may require increased dopaminergic tone to achieve similar levels of reinforcement and reward as C/C individuals. Studies have shown that one or two copies of the T allele of rs1800497 associates with disorders of reward deficiency such as alcohol dependence, smoking, and addictive behavior [14–17]. McAllister et al. found that rs1800497 allele status was associated with cognitive function following mild to moderate TBI (N=141: 93 TBI patients, 48 healthy controls) as defined by initial Glasgow Coma Scale (GCS) score of 9–15 and/or loss of consciousness (LOC) ≤24 h [11, 12]. The TBI group included 65 T-allele negative and 28 T-allele positive patients. T-allele positive patients showed worse episodic memory at 1 month post-TBI on the California Verbal Learning Test (CVLT) recognition trial, a result not observed in controls with the T allele. T-allele positive patients in the TBI group also exhibited slower performance on measures of response latency than those without the T allele [11, 12].

The present study extends this work in evaluating whether variation at rs1800497 within ANKK1 associates with verbal learning and non-verbal learning after acute TBI in a large multicenter cohort. We combined clinical and outcome data from two large prospective multicenter studies, The Citicoline Brain Injury Treatment Trial (COBRIT) [18, 19] and the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) [20] to create the largest sample size to date of adult TBI patients with rs1800497 genotyping and six-month outcome testing after acute TBI (N=492). The merging of these two large datasets was made possible by their shared common standards—the National Institute of Neurological Disorders and Stroke (NINDS) TBI Common Data Elements (CDEs) [21]. We tested the primary hypothesis that the rs1800497 associates with reduced performance on the CVLT as previously described by McAllister et al. [11, 12] and assessed secondary endpoints and tertiary endpoints including a measure of non-verbal processing (Wechsler Adult Intelligence Scale Processing Speed Index (WAIS-PSI)).

Materials and methods

Study design

COBRIT is a multicenter, two-group, phase three, double-blind randomized placebo-controlled clinical trial conducted at eight U.S. Level I Trauma Centers [18, 19]. Inclusion criteria were patients with blunt force trauma to the head requiring inpatient hospitalization for TBI, with either: (1) Glasgow Coma Scale (GCS) score 3–12 and GCS motor score <6, or (2) GCS 3–12 with motor score 6 *or* GCS 13–15 *or* paralyzed after administration of paralytics as part of the clinical course with ≥1 of the following CT parameters: ≥10-mm diameter intraparenchymal hemorrhage, ≥5-mm extra-axial hematoma, subarachnoid hemorrhage visible on two or more 5-mm slices, or midline shift ≥5 mm. TRACK-TBI Pilot is a multicenter prospective observational study with patients recruited through convenience sampling at three U.S. Level I Trauma Centers [20]. Inclusion criteria were external force trauma to the head and clinically indicated head CT scan within 24 h of injury.

Exclusion criteria for both studies included positive pregnancy test result or known pregnancy, imminent death or current life-threatening disease, incarceration, or evidence of serious psychiatric and neurologic disorders that interfere with outcome assessment. Non-English speakers were not enrolled due to inability to participate in outcome assessments, which are normed and administered in English. The COBRIT study also excluded patients with bilaterally fixed and dilated pupils, those with prior TBI requiring hospitalization, concurrent enrollment in another study, and/or acetylcholinesterase inhibitor use within two weeks prior to injury. One trauma center (University of Pittsburgh) participated in both COBRIT and TRACK-TBI Pilot, but patients at this site were not co-enrolled into both studies.

The institutional review boards of all participating sites approved the protocols for each study. Patients were approached for informed consent before enrollment. For patients unable to give consent, due to their injury, consent was obtained from their legally authorized representative (LAR). Patients consented by LAR were approached for informed consent to continue participation while in the hospital or during follow-up assessment time-points.

These two studies enrolled a large number of TBI patients through acute and intermediate care to provide an ethnically and demographically diverse patient population. In TRACK-TBI Pilot, a comprehensive acute clinical profile was obtained from each patient in accordance with the National Institutes of Health (NIH) and NINDS CDEs across demographics, medical history, injury characteristics, acute hospital clinical care, and neuroimaging [22–26]. Enrollment in COBRIT began prior to the release of the NIH NINDS CDEs, but variables were collected in a standardized fashion with a high degree of concordance with the CDE effort [18], which enabled data pooling between the two studies. The pharmacological intervention in COBRIT consisted of daily enteral/oral citicoline (2000 mg) or placebo for 90 days. As the primary report by Zafonte et al. in 2012 found no association between citicoline use and improvement in functional and cognitive outcome [19], we did not pursue outcome analysis between treatment and control arms for this study.

Patient selection

All adult patients with complete 6-month outcomes and an acute blood biospecimen drawn for DNA were selected for this analysis from the COBRIT and TRACK-TBI Pilot studies. In both studies, patients without genotyping results and/or complete 6-month outcomes were excluded. Of 1213 total adult patients in COBRIT, 739 patients did not have blood genotyping results available and 202 of the remaining 474 patients had no or incomplete outcomes, leaving a final N of 272 patients for analysis. Of 650 total patients in TRACK-TBI Pilot, 51 patients were excluded from the non-acute TBI site and 27 patients were under the age of 18. Of the remaining 572 adult patients, 166 did not have blood genotyping results and 186 had genotyping but no or incomplete outcomes, leaving a final N of 220 for analysis. A comparison between included and excluded adult patients for this analysis, by study, is discussed in the Results section and in Online Resource 1 and 2. The distributions of demographic and clinical descriptors for COBRIT patients by treatment group are summarized in Online Resource 3.

Blood collection and genotyping

Specimen acquisition was performed as previously described [20]. In brief, blood samples for DNA genotyping analysis were collected via peripheral venipuncture or existing peripheral venous indwelling catheters within 24 h of injury. Samples were collected in BD Vacutainer K_2-EDTA vacutainer tubes, and subsequently aliquoted and frozen in cryotubes at −80 °C within 1 h of collection in accordance with recommendations from the NIH-CDE Biomarkers Working Group [25]. DNA was extracted from isolated leukocytes using the Wizard® Genomic DNA Purification Kit as described by the manufacturer (Promega, Madison, WI). The ANKK1 C/T SNP (rs1800497) was genotyped utilizing TaqMan®SNP Genotyping Assay as described by the manufacturer (Applied Biosystems, Carlsbad, CA). Patients were categorized by genotype: T/T, C/T, or C/C.

Outcome measures

The primary outcome measure was the California Verbal Learning Test, Second Edition (CVLT-II) Trials 1-5 Standard Score [27], which is one of the "core" TBI CDE outcome measures and was collected in both COBRIT and TRACK-TBI Pilot [28, 29]. The CVLT-II is a verbal learning and memory task in which there are five learning trials, an interference trial, an immediate recall trial, and a post-20 min recall trial. The CVLT-II Trials 1–5 Standard Score (CVLT-TSS) is normed for age and sex, and provides a global index of verbal learning ability [27]. The Wechsler Adult Intelligence Scale processing speed index (WAIS-PSI) was used as a secondary outcome measure [30]. Tertiary outcome measures collected across both studies include the Glasgow Outcome Scale-Extended (GOSE) [31], the Satisfaction with Life Scale (SWLS) [32], the Trail Making Test (TMT) Trail B minus Trail A Score (TMT B-A) [33], and the Brief Symptom Inventory 18 (BSI18) Global Severity Index Score (BSI18 GSI) [34].

Statistical analysis

Primary analysis assessed the impact of the T allele (T/T, C/T, C/C) on the chosen cognitive outcome measures. Group differences in demographic and clinical descriptors across ANKK1 genotypes (T/T, C/T, C/C) were assessed by Pearson's chi-squared test (X^2) for categorical variables and analysis of variance (ANOVA) for continuous variables. Row categories with average cell counts of less than 5 by ANKK1 were combined into a single row category during analysis. Fisher's exact test was used for comparisons with more than 20 % of individual cell counts less than 5. A two-way ANOVA was performed to assess the main effects of ANKK1 dose and study cohort (COBRIT vs. TRACK-TBI) as well as their interaction on 6-month CVLT-TSS. If the interaction was not significant, significant main effects were confirmed with a two-way ANOVA omitting the interaction term, using Tukey's post hoc test with multiple-comparison correction. Fisher's permutation test [35] was performed as a sensitivity analysis to address the unequal distribution of ANKK1 across races. Fifty thousand permutations, within study and race, were used to evaluate the effect of ANKK1. Significance was assessed at $\alpha=0.05$ for all analyses. Fisher's permutation test was performed using Statistical Analysis System (SAS), Version 9.4 (SAS Institute, Cary, NC). All other analyses were performed using Statistical Package for the Social Sciences (SPSS), Version 21 (IBM Corporation, Chicago, IL).

Results

Demographic and clinical descriptors

A total of 492 patients were included in the analysis (COBRIT $N=272$ (55 %), TRACK-TBI Pilot $N=220$ (45 %)). The overall mean age was 40 years old (standard deviation (SD) 16), and subjects were 75 % male (Table 1). The overall race distribution was 78 % Caucasian, 15 % African American/African, and 2 % or less of each of the other races. Mechanisms of injury were 35 % fall, 24 % motor vehicle accident, 16 % motorcycle/bicycle accident, 13 % assault, 7 % pedestrian struck by vehicle, 3 % struck by/against object, and 2 % other. TBI classification by emergency department (ED) arrival GCS was as follows: 21 % severe (GCS 3–8), 8 % moderate (GCS 9–12), and 71 % mild (GCS 13–15).

Comparison by study demonstrated that there was a lower proportion of African/American-African patients and higher proportions of non-Caucasian, non-African-American/African patients in TRACK-TBI Pilot (Caucasian 75 %, African-American/African 11 %, other 14 %) than in COBRIT (80, 19, and 1 %, respectively, $p<0.001$). Mechanism of injury differed by study ($p<0.001$) with more falls (43 vs. 28 %), fewer motor vehicle accidents (19 vs. 28 %), and fewer motorcycle/bicycle accidents (10 vs. 21 %) observed in TRACK-TBI Pilot than in COBRIT, respectively. COBRIT patients presented with lower GCS (28 % severe, 10 % moderate, 62 % mild) than TRACK-TBI Pilot patients (12 % severe, 5 % moderate, 83 % mild, $p<0.001$). No differences by study were observed in age, gender, or ANKK1 genotype (Table 2).

ANKK1 genotype distribution

ANKK1 genotype distribution was 8 % T/T ($N=40$), 36 % C/T ($N=175$), and 56 % C/C ($N=277$) consistent with the HapMap Phase III average across all races [36]. ANKK1 allelic frequencies ($T=0.26$, $C=0.74$) were found to be at or near Hardy–Weinberg equilibrium ($p=0.263$, Pearson X^2). T

Table 1 Demographic and clinical descriptors by ANKK1 genotype

Baseline variable	All patients	T/T	C/T	C/C	Sig. (*p*)
Age	*N*=492	*N*=40	*N*=175	*N*=277	0.861
Mean±SD	40±16	39±13	40±16	41±16	
Gender	*N*=492	*N*=40	*N*=175	*N*=277	0.404
Male	366	31 (9 %)	124 (34 %)	211 (58 %)	
Female	126	9 (7 %)	51 (41 %)	66 (52 %)	
Race[a]	*N*=489	*N*=40	*N*=174	*N*=275	<0.001
African-American/African	76	16 (21 %)	32 (42 %)	28 (37 %)	
American Indian/Alaskan	2	2 (100 %)	0 (0 %)	0 (0 %)	
Asian	11	2 (18 %)	5 (45 %)	4 (36 %)	
Caucasian	380	18 (5 %)	128 (34 %)	234 (62 %)	
Hawaiian/Pacific Islander	9	1 (11 %)	4 (44 %)	4 (44 %)	
More than one race	11	1 (9 %)	5 (45 %)	5 (45 %)	
Mechanism of Injury[a]	*N*=491	*N*=40	*N*=174	*N*=277	0.106
Motor vehicle accident	118	9 (8 %)	49 (42 %)	60 (51 %)	
Motorcycle/bicycle accident	79	5 (6 %)	33 (42 %)	41 (52 %)	
Pedestrian struck by vehicle	33	2 (6 %)	14 (42 %)	17 (52 %)	
Fall	170	11 (6 %)	50 (29 %)	109 (65 %)	
Assault	66	10 (15 %)	17 (26 %)	39 (59 %)	
Struck by/against object	14	1 (7 %)	5 (36 %)	8 (57 %)	
Other	11	2 (18 %)	6 (55 %)	3 (27 %)	
ED arrival GCS	*N*=489	*N*=40	*N*=175	*N*=274	0.097
Mild (13–15)	348	34 (10 %)	125 (36 %)	189 (54 %)	
Moderate (9–12)	38	2 (5 %)	18 (47 %)	18 (47 %)	
Severe (3–8)	103	4 (4 %)	32 (31 %)	67 (65 %)	

Distribution of demographic and clinical descriptors by ANKK1 genotype. Row percentages are shown for categorical variables (may not equal exactly 100 % due to independent rounding). Statistical significance (*p*) is assessed using the Pearson chi-squared statistic or Fisher's exact test for categorical variables and ANOVA for continuous variables, by ANKK1 genotype with α=0.05. *ED* Emergency Department, *GCS* Glasgow Coma Scale

[a] Row categories with average cell counts of less than 5 are combined into a single row category during analysis

allele distribution differed across races ($p<0.001$) but conformed to known HapMap Phase III frequencies [36]. Distributions across the two primary race groups in this study were assessed: Caucasians (5 % T/T, 34 % C/T, 61 % C/C) did not differ from the expected CEU HapMap (5 % T/T, 28 % C/T, 66 % C/C ($p=0.291$)), and African American/Africans (21 % T/T, 42 % C/T, 37 % C/C) did not differ from the expected YRI HapMap (16 % T/T, 50 % C/T, 34 % C/C ($p=0.606$)). HapMap comparisons for ANKK1 were not performed for the other races due to small sample sizes of $n\leq 10$. No differences in ANKK1 genotype distribution were observed by age, gender, mechanism of injury, or GCS.

Comparison of descriptors between included and excluded patients by study

In both studies, there was a higher proportion of African-American/African patients included in this analysis (COBRIT *N*=270, 80 % Caucasian, 19 % African-American/African, 1 % other; TRACK-TBI Pilot *N*=220, 75 % Caucasian, 11 % African-American/African, 14 % other) compared to patients not included (COBRIT *N*=938, 83 % Caucasian, 13 % African-American/African, 4 % other, $p=0.033$; TRACK-TBI *N*=348, 85 % Caucasian, 6 % African-American, 9 % other, $p=0.043$). The included COBRIT patients had less severe injuries by GCS (*N*=271, 28 % severe, 11 % moderate, 62 % mild) compared to those not included (*N*=936, 39 % severe, 10 % moderate, 51 % mild, $p=0.004$). The included TRACK-TBI Pilot patients were younger (*N*=220, mean 41, SD 16) compared to those not included (*N*=352, mean 46, SD 19). No differences in other baseline descriptors or ANKK1 genotype distribution were observed between included and excluded adult patients within each study (Online Resource 1 and 2).

Comparison of descriptors between COBRIT treatment and control arms

The COBRIT patients included in this analysis (*N*=272) distributed evenly across citicoline (*N*=137 (50 %)) and placebo arms (*N*=135 (50 %)). No differences in any demographic and clinical descriptors were observed by treatment arm (Online Resource 3).

Table 2 Demographic and clinical descriptors by study

Baseline variable	COBRIT	TRACK-TBI Pilot	Sig. (*p*)
Age	*N*=272	*N*=220	0.453
Mean±SD	40±15	41±16	
Gender	*N*=272	*N*=220	0.072
Male	211 (78 %)	155 (70 %)	
Female	61 (22 %)	65 (30 %)	
Race[a]	*N*=270	*N*=219	<0.001
African-American/African	51 (19 %)	25 (11 %)	
American Indian/Alaskan	0 (0 %)	2 (1 %)	
Asian	2 (1 %)	9 (4 %)	
Caucasian	216 (80 %)	164 (75 %)	
Hawaiian/Pacific Islander	0 (0 %)	9 (4 %)	
More than one race	1 (0 %)	10 (5 %)	
Mechanism of injury	*N*=272	*N*=219	<0.001
Motor vehicle accident	77 (28 %)	41 (19 %)	
Motorcycle/bicycle accident	58 (21 %)	21 (10 %)	
Pedestrian struck by vehicle	13 (5 %)	20 (9 %)	
Fall	76 (28 %)	94 (43 %)	
Assault	33 (12 %)	33 (15 %)	
Struck by/against object	8 (3 %)	6 (3 %)	
Other	7 (3 %)	4 (2 %)	
ED arrival GCS	*N*=271	*N*=218	<0.001
Mild (13–15)	168 (62 %)	180 (83 %)	
Moderate (9–12)	27 (10 %)	11 (5 %)	
Severe (3–8)	76 (28 %)	27 (12 %)	
ANKK1 genotype	*N*=272	*N*=220	0.193
T/T	17 (6 %)	23 (11 %)	
C/T	102 (38 %)	73 (33 %)	
C/C	153 (56 %)	124 (56 %)	

Distribution of demographic and clinical descriptors by study. Column percentages are shown for categorical variables (may not equal exactly 100 % due to independent rounding). Statistical significance (*p*) is assessed using the Pearson chi-squared statistic or Fisher's Exact Test for categorical variables, and ANOVA for continuous variables, by ANKK1 genotype with α=0.05. *ED* Emergency Department, *GCS* Glasgow Coma Scale

[a] Row categories with average cell counts of less than 5 are combined into a single row category during analysis

Relationship of ANKK1 to CVLT-TSS

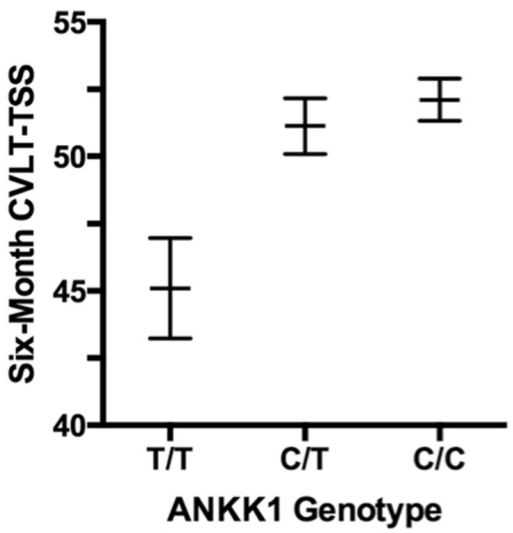
Our analyses were designed to address potential confounding created by pooling COBRIT and TRACK-TBI Pilot data for the effects of the following: (1) the particular study and (2) interaction between ANKK1 and the particular study on CVLT-TSS. First, we performed a two-way ANOVA with CVLT-TSS as the dependent variable to assess the main effects of ANKK1 and study, plus the interaction term ANKK1 X study. Table 3 shows that ANKK1 had a statistically significant association at α=0.05 with CVLT-TSS ($F(2, 486)=4.964$, $p=0.007$), while particular study and ANKK1 X study did not. We then re-ran the model, omitting the interaction term, to confirm the significant association between ANKK1 and CVLT-TSS ($F(2,486)=4.893$, $p=0.008$), and not between particular study and CVLT-TSS ($F(1,486)=0.117$, $p=0.732$). We performed Tukey's post-hoc test for ANKK1 in the same model to assess for differences in CVLT-TSS across the three ANKK1 genotypes. Figure 1

Table 3 Association of ANKK1 genotype and study with 6-month CVLT-TSS

Source	Type III sum of squares	*df*	Mean square	*F*	*p* value
Corrected model	1773.6	5	354.7	1.993	0.078
ANKK1	1767.4	2	883.7	4.964	0.007
Study	54.2	1	54.2	0.304	0.581
ANKK1 X study	35.0	2	17.5	0.098	0.906
Error	86517.8	486	178.0		

Two-way ANOVA to assess the main effects of ANKK1 genotype (abbreviated as ANKK1) and Study (COBRIT or TRACK-TBI Pilot) plus the interaction term (ANKK1 X Study) on six-month CVLT-TSS as the dependent variable in the model. Significant assessed at α = 0.05

CVLT-TSS California Verbal Learning Test, Second Edition Trials 1-5 Standard Score, *df* degrees of freedom



ANKK1 Genotype (I vs. J)	Mean CVLT-TSS (SE) (I vs. J)	MD (95% CI) (I minus J)	p-value
T/T vs. C/T	45.1 (1.9) vs. 51.1 (1.0)	-6.0 (-11.5, -0.5)	0.027
T/T vs. C/C	45.1 (1.9) vs. 52.1 (0.8)	-7.0 (-12.3, -1.7)	0.006

Fig. 1 Comparison of 6-month CVLT-TSS means across ANKK1 genotypes. Graph shows 6-month CVLT-TSS mean±SE by ANKK1 genotype. Tukey's post hoc test was used to assess mean differences (MD) in CVLT-TSS between genotypes. Only significant MDs at α=0.05 are shown in the table. Mean difference is calculated by the mean CVLT-TSS of the first genotype (I) minus that of the second genotype (J). *CVLT-TSS* California Verbal Learning Test, Second Edition Trials 1–5 Standard Score, *SE* standard error, *CI* confidence interval

shows the CVLT-TSS means by ANKK1, and that mean CVLT-TSS of T/T patients differed significantly from that of C/T and C/C patients, with a mean decrease of 6.0 points against C/T and 7.0 points against C/C.

Based on our initial descriptive statistics (Table 1), there were subpopulation differences in the distribution of ANKK1 genotypes across races. As a sensitivity analysis, we ran Fisher's permutation test as a distribution-free alternative to the parametric model [35]. The association between ANKK1 and six-month CVLT-TSS remained significant (p=0.026) when controlling for race and particular study.

Exploratory analysis of ANKK1 on other outcome measures

To explore the common six-month outcome measures in our pooled multicenter dataset, we assessed the association between ANKK1 genotype on a non-verbal cognitive test, the WAIS-PSI, as well as with four other measures: GOSE, SWLS, TMT B-A, BSI18 GSI. We performed identical analyses as above to assess the main effect of ANKK1 genotype and particular study, plus the interaction factor ANKK1 X study, using two-way ANOVA with each outcome measure as the dependent variable. There was a significant association at α=0.05 between ANKK1 and WAIS-PSI (F(2,486)=3.225, p=0.041), and particular study and WAIS-PSI (F(1,486)=7.01, p=0.008), with no effect of ANKK1 X study. No significant pairwise differences at α=0.05 were observed in WAIS-PSI means across ANKK1 (T/T: 94.1, SE 2.5; C/T: 95.9, SE 1.3; C/C: 98.8; SE 0.9) on Tukey's post-hoc test. Mean WAIS-PSI scores in COBRIT were lower than in TRACK-TBI Pilot (COBRIT: 95.9, SE 1.0; TRACK-TBI Pilot 99.3, SE 1.1, p=0.02). There was no significant association between ANKK1, study or ANKK1 X study with GOSE, SWLS, or TMT B-A. There was a marginal association at α=0.05 between ANKK1 and BSI18 GSI (F(2,486)=3.0, p=0.052), with no effect of particular study or ANKK1 X study. On Tukey's post hoc test, BSI18 GSI means (T/T 60.1, SE 2.1; C/T 54.7, SE 0.9; C/C 56.3, SE 0.7) differed significantly at α=0.05 between T/T and C/T only (95 % CI 0.3 to 10.4, p=0.036).

Discussion

Over the past decade, genetic association studies have contributed to our understanding of the molecular mechanisms of multiple common human diseases, including Alzheimer disease, heart disease, and diabetes, among others [37–42]. In each case, molecular mechanisms suspected to be involved in disease pathogenesis based on preclinical or pathologic studies were confirmed by human genetics. In addition, human genetic association studies have uncovered new molecular pathways previously unsuspected to play a role in disease pathogenesis [43–47]. The overwhelming majority of these genetic discoveries, however, have applied to disease risk [48–52]. TBI presents special challenges for genetic association studies [53]. First, there is a prominent and stochastic environmental factor: the traumatic injury. Second, premorbid personality and developmental factors play a clear role in recovery from injury. Thus, in order to identify molecular pathways in resilience to or recovery from TBI, large sample sizes and collection of comprehensive data, which allow for consideration of premorbid factors and assessment of injury severity, are essential [54, 55]. The use of CDEs is fundamental to the success of these efforts and the NIH-NINDS TBI CDEs were designed to address this need [21]. Investigators of COBRIT and TRACK-TBI Pilot were among the leaders in this effort, and the present study was feasible because of the high degree of overlap between the assessment tools and outcome measures utilized in the two studies.

Our robust sample permitted confirmation of the hypothesis concerning the effects of the T allele on cognitive outcome. Indeed, we found an association between ANKK1 and poorer performance on 6-month CVLT-TSS specifically tied to the T/T genotype. The C/T group alone did not show any differences from the C/C group on CVLT-TSS. Although this does not align perfectly with previous findings in TBI, where T-allele carriers showed worse performance on an episodic memory task of the CVLT, our overall result remains more confirmatory than divergent. McAllister et al. reported only one T/T individual in a sample size of 141, which could not enable a T-dose-dependent analysis. The distribution ANKK1 genotypes in our analysis approaches that of the general population according to HapMap Phase III and therefore allows us more statistical power to investigate the differential relationships between genotype and cognition. Secondly, it may be that differential genotypic associations with specific symptoms are more easily identified on specialized verbal memory trials such as the CVLT recognition task while the deleterious effect of a double dose of T allele manifest on the CVLT-TSS, a more highly generalizable and normative global index of verbal learning ability.

Our study reinforces the benefits of pooling multicenter trials into a unified data commons. There were no differential study effects by COBRIT and TRACK-TBI Pilot, nor were there ANKK1 X study interactions, on six-month verbal learning. This validates data sharing as a mechanism to raise statistical power for hypothesis testing and increases our confidence in the associations of ANKK1 T/T with verbal learning across a large, heterogeneous TBI population.

As well, merging COBRIT and TRACK-TBI Pilot data effectively captures patients across the entire TBI spectrum. As COBRIT excluded patients with GCS 13–15 presenting with negative head CTs, it targeted patients with more moderate and severe TBI whereas TRACK-TBI Pilot enrolled patients with similar TBI incidence as reported in literature and the

population, which is predominantly mild [56, 57]. Indeed, COBRIT patients in the current analysis presented with more severe TBI compared to TRACK-TBI Pilot, and this difference may account for the observed differences by study in some of our analyses of secondary outcomes. For example, the study effect on WAIS-PSI scores reached significance. It is also interesting to note the marginal signal of ANKK1 T/T with the BSI18 GSI, which corroborates the range of studies interrogating ANKK1 in the context of neuropsychiatric disorders.

Our study has clarified several key areas identified by McAllister et al. as areas of further investigation concerning the relationship of ANKK1 with TBI outcome [11, 12]. The authors questioned whether their results would hold in a larger, more diverse racial and ethnic population, with varying injury severity and in outcomes at a longer post-injury interval. By utilizing two multicenter studies (COBRIT: eight centers, TRACK-Pilot: three centers, one center participated in both studies), the sample size was expanded to encompass a total of 10 Level I trauma centers across the USA. This heterogeneous population covers the full severity spectrum from concussion to coma, which previous studies did not have an opportunity to evaluate in the context of ANKK1. Regarding outcomes, McAllister et al. were only able to access CVLT at 1-month post-injury and expressed concern about generalizability at later timepoints. With a larger multicenter sample and long-term follow-up (the 6-month clinical standard), the present study is more resilient to local demographic and practice effects providing a strong replication test of McAllister et al.'s results.

Limitations

Although we have improved upon the breadth and generalizability of previous studies, we recognize several limitations in the current analysis. First, we could not fully account for the impact of TBI pathology and lesion types on recovery, the lack of pre-injury psychometric tests, other genetic predispositions, and non-TBI control groups. As our primary analysis was confirmatory in nature, we pursued similar inclusion criteria as McAllister et al. for general TBI and did not explore the structure-function implications of ANKK1 with intracranial lesion types or baseline mental health variables. Given the heterogeneity of TBI, subjects may never be perfectly matched by type, location, and extent of injury. Despite this fact, convincing evidence of genetic association can be clarified by sufficiently large sample sizes. The ability to comment on causative or confounding relationships between ANKK1 and pre- or post-injury risk factors is beyond the scope of the current analysis. As T/T has been associated with propensity for addiction and poor coping strategies [8, 14–17, 58, 59], the acquisition and analysis of detailed pre-injury addictive behavior, post-acute treatment, and recovery variables are relevant next steps in delineating the contribution of ANKK1 to both TBI risk and outcome variability. We are also constrained by the lack of genome-wide data, which makes it difficult to fully control for population stratification, as evidenced by the observed differences for patients who met the inclusion criteria for this analysis compared their excluded counterparts in COBRIT and TRACK-TBI Pilot. The proportion of T/T within our sample is still rather small, limiting our ability to assess whether there is a differential influence of ANKK1 genotypes on other domains of outcome, or in different races. The robustness of the association between ANKK1 and a given outcome domain such as working memory or processing speed, which encompasses multiple individual outcome measures, can be interrogated using multivariate integration and correlated with specific injuries in the dorsolateral prefrontal cortex—where working memory processes are known to be confined [60–62]. Work of this type is ongoing in the TRACK-TBI consortium.

In analyzing patients with full outcomes, there is an inherent risk of selecting for patients able to return for follow-up. For example, in our study, the COBRIT patients with genotyping and complete six-month outcomes presented with less severe injuries than those who had incomplete outcomes. This may be attributable in part to better cognition and functional ability to return for follow-up. As observed in TRACK-TBI Pilot, patients of younger age may be more mobile and/or available to return for full outcomes assessment. In some ways, the selection bias relates to the primary goal of this analysis, which was to assess the association of ANKK1 with outcome measures common to both studies and hence contingent on patients with valid scores. It is difficult to capture reasons for incomplete outcomes in patients who are lost to follow-up, as in many cases contact is never made.

The molecular mechanism and active location of ANKK1 remains a topic of ongoing study, with further experiments needed in cellular and animal models, as well as human trials. There is a need to examine gene-gene interaction with other loci of susceptibility for prognostic phenotyping within the dopaminergic system to elucidate an ANKK1 molecular pathway in local CNS physiology, contingent on detailed structure-function analysis from the comprehensive mapping of the human connectome [63]. Alternatives to the limitations of conventional imaging modalities such as CT are being explored with TRACK-TBI Pilot data. Early results indicate that prediction models including contusion on 3T MRI and axonal injury by diffusion tensor imaging (DTI) surpass other predictors for global outcome prediction in a subset of patients after mild TBI [64]. Advanced diffusion imaging modalities targeting the dorsal prefrontal cortex have been reported for healthy and diseased states [65–69]. Increased precision in characterizing regional pathophysiology will enable more objective control of injury type and severity in order to distill the specific mechanism by which ANKK1 modulates working memory, as a subset of the disparate patterns of cognitive impairment observed in the current TBI classification system of mild, moderate, and severe. In a broader sense, further

development of classification approaches based on quantitative morphometry [70], in conjunction with appropriate computational methods [71] and data integration processes [72], will aid in deconstructing the contribution of genetic modulation to multidimensional domains of outcome after TBI.

Greater sample size and more extensive genotyping will overcome our current limitations to allow for stratification across known genetic profiles and TBI severities, as well as raise statistical power to levels appropriate for phase III clinical trials. We successfully pooled COBRIT and TRACK-TBI Pilot data through outcome measures common to both studies, but we were still constrained in our scope of data pooling. Clearer evaluations of the effects of risk factors and predictors of TBI outcome, including ANKK1 and other SNPs, await the expanded initiatives of current multicenter studies such as the Transforming Research and Clinical Knowledge in TBI study (TRACK-TBI) [73] and the Collaborative European NeuroTrauma Effectiveness Research in TBI study (CENTER-TBI) [74], which will enroll 3000 and 5000 patients with controls, respectively, over the next five years, using the expanded Version 2 of the NIH-NINDS TBI CDEs [21, 75]. Adopting an international approach [76] to this standardized set of variables with wide scope, utility, and applicability will allow us to converge and leverage research efforts to achieve the sample sizes we truly need for delineating the effects of the ANKK1 polymorphism in TBI.

Conclusions

In the largest prospective multicenter study to date examining the incidence of the rs1800497 SNP in TBI, enabled by data pooling of shared common variables, we report that the ANKK1 T/T genotype associates with poorer verbal learning performance on CVLT-TSS at six months post-injury across the spectrum of TBI severity. With the augmented statistical power of this analysis, successful replication of the association between ANKK1 and cognition reinforces the potential implication of a DRD2-dependent biological mechanism underlying cognitive performance after TBI.

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Compliance with Ethical Standards

Disclosure of Potential Conflicts of Interest The authors declare that they have no conflicts of interest.

Research Involving Human Participants All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

DRD2 C957T polymorphism is associated with improved 6-month verbal learning following traumatic brain injury

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Abstract Traumatic brain injury (TBI) often leads to heterogeneous clinical outcomes, which may be influenced by genetic variation. A single-nucleotide polymorphism (SNP) in the dopamine D2 receptor (*DRD2*) may influence cognitive deficits following TBI. However, part of the association with *DRD2* has been attributed to genetic variability within the adjacent ankyrin repeat and kinase domain containing 1 protein (*ANKK1*). Here, we utilize the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) study to investigate whether a novel *DRD2 C957T* polymorphism (rs6277) influences outcome on a cognitive battery at 6 months following TBI—California Verbal Learning Test (CVLT-II),

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Wechsler Adult Intelligence Test Processing Speed Index Composite Score (WAIS-PSI), and Trail Making Test (TMT). Results in 128 Caucasian subjects show that the rs6277 T-allele associates with better verbal learning and recall on CVLT-II Trials 1–5 (T-allele carrier 52.8 ± 1.3 points, C/C 47.9 ± 1.7 points; mean increase 4.9 points, 95% confidence interval [0.9 to 8.8]; $p = 0.018$), Short-Delay Free Recall (T-carrier 10.9 ± 0.4 points, C/C 9.7 ± 0.5 points; mean increase 1.2 points [0.1 to 2.5]; $p = 0.046$), and Long-Delay Free Recall (T-carrier 11.5 ± 0.4 points, C/C 10.2 ± 0.5 points; mean increase 1.3 points [0.1 to 2.5]; $p = 0.041$) after adjusting for age, education years, Glasgow Coma Scale, presence of acute intracranial pathology on head computed tomography scan, and genotype of the *ANKK1* SNP rs1800497 using multivariable regression. No association was found between *DRD2 C947T* and non-verbal processing speed (WAIS-PSI) or mental flexibility (TMT) at 6 months. Hence, *DRD2 C947T* (rs6277) may be associated with better performance on select cognitive domains independent of *ANKK1* following TBI.

Keywords Traumatic brain injury · Genetic factors · Cognition · Outcome measures · Human studies

Introduction

Traumatic brain injury (TBI) is a significant source of morbidity and mortality—an estimated 2.5 million cases occur annually in the USA alone [1] Initial injury severity is commonly stratified into severe, moderate, and mild TBI categories as defined by an initial Glasgow Coma Scale (GCS) score of 8 or less, 9 to 12, and 13 to 15, respectively [2, 3]. Individuals with similar injuries often follow divergent clinical trajectories [4]. Up to 5.3 million people live with long-term disability from TBI, and numerous others experience persistent TBI-related sequelae—including cognitive deficits, changes in personality, and increased rates of post-traumatic psychiatric disorders such as depression and/or post-traumatic stress disorder [5, 6]. However, factors influencing variability in post-traumatic clinical course remain unclear and efforts are needed to better identify those at greatest risk for post-traumatic sequelae [7].

Studies have begun to suggest that genetic variability—such as single-nucleotide polymorphisms (SNPs)—may be one factor which contributes to observed clinical variance. A number of polymorphisms influencing protein structure, function, and/or availability have been identified [8–11]. In particular, SNPs arising within the dopaminergic system may influence cognition and cognitive recovery following TBI [12]. The neurotransmitter dopamine is essential for proper neuronal function of the striate nucleus linked to learning and memory [13]. One important molecular component of dopaminergic signaling pathways is the dopamine D2 receptor (DRD2), which is highly expressed in the striatum of the subcortical forebrain. DRD2 binds to dopamine in the synaptic cleft and initiates post-synaptic secondary messenger cascades, which modulate neuronal circuits contributing to several cognitive domains, namely learning [14]. Reduced DRD2 expression has been linked to cognitive impairment and psychiatric disease [15, 16]. Furthermore, stimulation of DRD2 in the striatum has been shown to potentiate learning when treated with a D2-specific agonist [13, 17].

Given the prevalence of cognitive defects in TBI patients, there is an interest in identifying SNPs that associate with poor cognitive outcome [13, 18]. The *DRD2* gene is located on chromosome 11 q22–23 with a relatively common SNP located within exon 7 with a single-nucleotide cytosine to thymine substitution—known as the *C957T* SNP rs6277 [19, 20]. This substitution has been associated with decreased affinity of the striatal D2 receptors [21] and is associated with better learning, verbal memory, and cognitive ability in the psychiatry literature [22–24]. Initial studies report a potential connection between *DRD2 C957T* and cognitive performance following TBI [13, 15, 18]. However, this observation may be confounded by linkage effects with ankyrin repeat and kinase domain containing 1 protein (*ANKK1*) *TaqIA (rs1800497)*—a gene adjacent to and oriented tail to tail with *DRD2* on chromosome 11 [13, 25]. Therefore, a potential modulatory role of *DRD2 C957T* on cognitive performance remains unclear and warrants further investigation.

For the current analysis, we utilized data from the prospective multicenter Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Pilot study in order to explore associations between the *DRD2 C957T* SNP and cognitive outcomes post-TBI while controlling for *ANKK1 Taq1A* [26]. We demonstrate that the *DRD2 C957T* T-allele is associated with better performance on verbal memory but not processing speed or mental flexibility at 6 months post-TBI.

Methods

Study design

The TRACK-TBI Pilot Study is a multicenter prospective observational study conducted at three level I trauma centers in the USA—San Francisco General Hospital, University of Pittsburgh Medical Center, and University Medical Center Brackenridge (UMCB) in Austin, TX—using the National Institutes of Health (NIH) and National Institute of Neurological Disorders and Stroke (NINDS) common data elements (CDEs) [26–30]. Inclusion criteria for the pilot study were adult patients presenting to a level I trauma center with external force trauma to the head and clinically

indicated head computed tomography (CT) scan within 24 h of injury. Exclusion criteria were pregnancy, comorbid life-threatening disease, incarceration, on psychiatric hold, and non-English speakers due to limitations in participation with outcome assessments. For the present study, our goal was to study the association of the *DRD2 C957T* polymorphism on cognitive outcome after TBI uncomplicated by massive intracranial injury, neurosurgical intervention, or polytrauma. Therefore, our analysis was restricted to a subset of adult patients with Marshall CT Score 1–2; no acute neurosurgical intervention; no developmental delay; and no severe, critical, or unsurvivable extracranial injuries as defined by an Abbreviated Injury Scale (AIS) score >3 in any extracranial body region. Due to the small numbers and unequal distribution of *DRD2 C957T* genotypes in other races in our sample, all selected patients were of Caucasian race.

Eligible subjects were enrolled through convenience sampling at all three sites. Institutional review board approval was obtained at all participating sites. Informed consent was obtained for all subjects prior to enrollment in the study. For patients unable to provide consent due to their injury, consent was obtained from their legally authorized representative (LAR). Patients were then reconsented, if cognitively able at later inpatient and/or outpatient follow-up assessments for continued participation in the study.

Biospecimen acquisition and genotyping

Specimen acquisition was performed as previously described [30]. In brief, blood samples for DNA genotyping analysis were collected via peripheral venipuncture or existing peripheral venous indwelling catheters within 24 h of injury. Samples were collected in BD Vacutainer K_2-EDTA Vacutainer tubes and subsequently aliquoted and frozen in cryotubes at −80 °C within 1 h of collection in accordance with recommendations from the NIH-CDE Biomarkers Working Group [29]. DNA was extracted from isolated leukocytes using the Wizard® Genomic DNA Purification Kit as described by the manufacturer (Promega, Madison, WI). The *DRD2 C957T* (rs6277) and *ANKK1 TaqIA* (rs1800497) polymorphisms were genotyped using the TaqMan® SNP Genotyping Assay as described by the manufacturer (Applied Biosystems, Carlsbad, CA; rs6277 Assay ID# C__11339240_10; rs1800497 Assay ID# C__7486676_10). For the purposes of evaluating a potential protective benefit of the *DRD2 C957T* T-allele, C/T and T/T individuals were combined as a single group as previously described for *DRD2 C957T* [13, 22, 23]. Therefore, for data recording and all figures, this group is referred to as *DRD2 C957T* T-Present. Likewise, *ANKK1 TaqIA* genotype was dichotomized by T-allele carriers versus non-carriers as described previously [13].

Neuropsychiatric testing and outcome parameters

The NINDS defines measures of neuropsychological impairment as those "of neuropsychological functions, such as attention, memory, and executive function which are very sensitive to effects of TBI that affect everyday activities and social role participation." To evaluate for neuropsychological impairment, all participants underwent outcome assessment at 6 months following TBI with a battery of NIH NINDS-designated "Core Measures"—those deemed most relevant and applicable across large TBI studies. For the current analysis, all three measures of the "neuropsychological impairment" domain of the outcome CDEs were included.

California Verbal Learning Test, second edition

The California Verbal Learning Test (CVLT)-II is a verbal learning and memory task in which five learning trials, an interference trial, an immediate recall trial, and a post-20-min recall trial are performed. The CVLT-II was substituted for the Rey Auditory Verbal Learning Test (RAVLT) listed in the NIH NINDS outcome CDEs, due to relevant revisions of the second edition and higher consistency on between-norm sets as previously described [31, 32]. The CVLT-II Trial 1–5 raw score provides a global index of verbal learning ability [33]. Further, lower scores on the CVLT-II Short-Delay Free Recall (SDFR) indicate retroactive interference, while lower scores on the CVLT-II Long-Delay Free Recall (LDFR) indicate the occurrence of rapid forgetting. As outlined, all CVLT raw scores are adjusted for age and years of education as part of the current analysis [33].

Wechsler Adult Intelligence Scale, fourth edition, Processing Speed Index Subscale

The Wechsler Adult Intelligence Test Processing Speed Index Composite Score (WAIS-PSI) is composed of two non-verbal tasks (symbol search and coding) which require visual attention and motor speed [34]. The composite score, normalized for age, was used in this analysis. On this test, a higher score reflects improved non-verbal processing speeds. In prior versions of this test, WAIS III, TBI has demonstrated that the WAIS-PSI predominately reflects impairment in perceptual processing speed with a small component attributable to working memory and only minimal contribution from motor speed [35]. The WAIS-PSI composite score includes adjustment for age and thus is adjusted only for years of education as part of the current analysis [34].

Trail Making Test

The Trail Making Test (TMT) is a two-part timed test (TMT-A and TMT-B). TMT-A assesses visual processing, and TMT-B

assesses mental flexibility and processing speed [36]. In order to increase the accuracy of the score with respect to the flexibility and processing speed without accounting for visual processing, we subtracted the first trial from the second trial (TMT B-A) as previously described [37]. On this test, a lower score suggests improved performance. The TMT B minus A score is adjusted for age and years of education as part of the current analysis [36].

Statistical analysis

Descriptive variables are presented as means and standard deviations (SDs) for continuous variables and as proportions for categorical variables. Group differences in patient demographics and injury characteristics across *DRD2 C957T* genotypes were assessed by Pearson's chi-squared test (X^2) for categorical variables and analysis of variance (ANOVA) for continuous variables. Fisher's exact test was used to assess for differences in categorical variables with individual cell counts ≤5. Linear regression was performed to assess the univariate association between *DRD2 C957T* genotype and each of the five outcome measures, adjusted for age and education years for CVLT measures and TMT B-A, and for education years only for WAIS-PSI, as described in the respective "Methods" section previously. Multivariable linear regression was performed to adjust for *ANKK1 TaqIA* genotype, gender, post-traumatic amnesia, emergency department admission GCS, and intracranial pathology on initial head CT scan for each outcome measure. The adjusted means and standard errors (SE) are reported for *DRD2 C957T* genotypes, and the adjusted mean differences (B) and their associated 95% confidence intervals (CI) are reported for predictors in each regression analysis. Significance was assessed at $\alpha = 0.05$. All analyses were performed using Statistical Package for the Social Sciences (SPSS) v. 22 (IBM Corporation, Chicago, IL).

Results

Demographic and injury characteristics

In total, 128 subjects were included in the current analysis (Table 1). The majority were male (64%) and all self-identified as Caucasian. Mean age was 44.4 ± 16.4 years, and mean years of education were 14.3 ± 2.7. Mechanisms of injury included fall (50%), motor vehicle accident (25%), pedestrian versus automobile (13%), assault (10%), and struck by object (2%). Mean GCS was 13.5 ± 3.2. Injury severity by admission GCS was 85% mild, 5% moderate, and 10% severe TBI. Thirty-two percent of patients did not have post-traumatic amnesia, while 56% had positive amnesia and 12% were unknown. Thirty-eight percent of patients showed positive intracranial pathology on initial head CT. *DRD2 C947T* (rs6277) was distributed with the following *n*s: C/C = 42, C/T = 58, and T/T = 28 (C-allele frequency 0.55, T-allele frequency 0.45), conforming to the Hardy-Weinberg equilibrium ($X^2 = 0.88$, $p > 0.05$) and known Caucasian-European (CEU) HapMap distribution (C-allele frequency 0.53, T-allele frequency 0.47). No statistically significant differences were observed for any demographic or clinical descriptor across *DRD2 C957T* genotypes (Table 1). *ANKK1 TaqIA* (rs1800497) was distributed with the following *n*s: C/C = 79, C/T = 42, and T/T = 7 (C-allele frequency 0.78, T-allele frequency 0.22), conforming to the Hardy-Weinberg equilibrium ($X^2 = 0.20$, $p > 0.05$) and known CEU HapMap distribution (C-allele frequency 0.81, T-allele frequency 0.19). The *ANKK1 TaqIA* polymorphism distributed differently across *DRD2 C957T*; 26/86 (30%) of *DRD2 C957T* T-allele carriers, versus 23/42 (55%) of *DRD2* C/C individuals, carried the *ANKK1* T-allele ($p = 0.007$); the lower concurrent inheritance of *DRD2 C957T* T-allele and the *ANKK1 TaqIA* T-allele is consistent with prior reports [25, 38].

DRD2 C957T is associated with verbal memory but not processing speed or mental flexibility

We first sought to characterize whether the *DRD2 C957T* polymorphism was associated with global or domain-specific differences in 6-month cognitive performance. *DRD2 C957T* T-allele carriers were found to perform better on CVLT-II Trials 1–5 (mean increase 4.4 points, 95% CI [0.4 to 8.5], $p = 0.033$); a non-significant statistical trend was found for CVLT-II Short-Delay Free Recall (mean increase 1.1 points, 95% CI [−0.1 to 2.4], $p = 0.073$) and Long-Delay Free Recall (mean increase 1.1 points, 95% CI [−0.1 to 2.4], $p = 0.083$). No differences were found for TMT B-A ($B = -13.6$, 95% CI [−31.3 to 4.1], $p = 0.131$) or WAIS-PSI ($B = 1.3$, 95% CI [−4.2 to 6.8], $p = 0.639$) (Table 2). These data suggest that the *DRD2 C957T* polymorphism is not associated with a global improvement in cognitive performance, but rather a specific performance advantage with tasks of verbal learning and recall.

DRD2 C957T is associated with verbal memory after multivariable correction

We next sought to evaluate whether the association between the *DRD2 C957T* polymorphism and CVLT-II performance persisted after adjusting for known predictors of outcome after TBI. For each of the five outcome measures, *DRD2 C957T* was entered into a multivariable model including *ANKK1 TaqIA* genotype, gender, presence/absence of post-traumatic amnesia, admission GCS, and presence/absence of intracranial pathology on CT in addition to age and education years.

On multivariable analysis of CVLT-II Trials 1–5, the *DRD2* T-allele is associated with improved performance compared to

Table 1 Demographic and clinical characteristics of included patients, by *DRD2 C957T* genotype

Variable	Overall ($N = 128$)	T-Present ($N = 86$)	T-Absent ($N = 42$)	Sig. (p)
Age (years)				
Mean, SD	44.4 ± 16.4	45.1 ± 16.7	43.1 ± 15.9	0.527
Gender				
Male	82 (64%)	57 (66%)	25 (60%)	0.455
Female	46 (36%)	29 (34%)	17 (40%)	
Education (years)				
Mean, SD	14.3 ± 2.7	14.4 ± 2.8	14.0 ± 2.7	0.460
Mechanism of injury				
Motor vehicle crash	32 (25%)	22 (26%)	10 (24%)	0.964
Pedestrian versus auto	26 (13%)	11 (13%)	5 (12%)	
Fall	64 (50%)	42 (49%)	22 (54%)	
Assault	13 (10%)	8 (9%)	5 (12%)	
Struck by	2 (2%)	2 (2%)	0 (0%)	
Post-traumatic amnesia				
No	41 (32%)	30 (35%)	11 (26%)	0.371
Yes	72 (56%)	48 (56%)	24 (57%)	
Unknown	15 (12%)	8 (9%)	7 (17%)	
ED arrival GCS				
Mean, SD	13.5 ± 3.2	13.6 ± 3.2	13.4 ± 3.3	0.756
Severe (3–8)	13 (10%)	10 (12%)	3 (7%)	
Moderate (9–12)	6 (5%)	1 (1%)	5 (12%)	
Mild (13–15)	109 (85%)	75 (87%)	34 (81%)	
CT intracranial pathology				
No	79 (62%)	54 (63%)	25 (60%)	0.721
Yes	49 (38%)	32 (37%)	17 (40%)	
ANKK1 TaqIA genotype				
T-Present	49 (38%)	26 (30%)	23 (55%)	0.007
T-Absent	79 (62%)	60 (70%)	19 (45%)	

All distributions are reported as column percentages

CT computed tomography, *DRD2* dopamine receptor D2, *ED* emergency department, *GCS* Glasgow Coma Scale, *SD* standard deviation

non-carriers as evidenced by a mean increase of 4.9 points (95% CI [0.9 to 8.8], $p = 0.018$) (Table 3). Male gender showed a mean decrease of 4.0 points (95% CI [−7.8 to −1.1], $p = 0.044$), and CT-positive patients had a mean decrease of 5.8 points (95% CI [−10.0 to −1.6], $p = 0.007$). *ANKK1* genotype, post-traumatic amnesia, and admission

Table 2 Adjusted univariate analysis of 6-month cognitive performance, by *DRD2 C957T* genotype

Outcome measure	T-Present ($N = 86$)	T-Absent ($N = 42$)	B [95% CI]	F-ratio	Sig. (p)
CVLT-II Trials 1–5[a]	52.1 (1.2)	47.6 (1.7)	4.4 [0.4, 8.5]	4.66	0.033
CVLT-II Short-Delay Free Recall[a]	10.8 (0.4)	9.7 (0.5)	1.1 [−0.1, 2.4]	3.27	0.073
CVLT-II Long-Delay Free Recall[a]	11.5 (0.4)	10.4 (0.5)	1.1 [−0.1, 2.4]	3.06	0.083
TMT Trail B minus A time[b]	49.5 (5.1)	63.1 (7.3)	−13.6 [−31.3, 4.1]	2.31	0.131
WAIS-PSI composite score[a]	100.5 (1.6)	99.2 (2.3)	1.3 [−4.2, 6.8]	0.22	0.639

Distributions are reported as mean ± standard error of the raw score for each cognitive measure, adjusted for age and education years for CVLT-II Trials 1–5, Short-Delay Free Recall, Long-Delay Free Recall, and TMT; WAIS-PSI composite score is adjusted for education years, as it is already normed for age

CVLT California Verbal Learning Test, *TMT* Trail Making Test, *WAIS-PSI* Wechsler Adult Intelligence Scale, Fourth Edition Processing Speed Index

[a] Higher scores suggest improved performance

[b] Lower scores suggest improved performance

Table 3 Multivariable analysis of 6-month verbal memory performance by *DRD2 C957T* genotype

Predictor	T-Present	T-Absent	*B* [95% CI]	*F*-ratio	Sig. (*p*)
CVLT-II Trials 1–5					
DRD2 C957T	52.8 ± 1.3	47.9 ± 1.7	4.9 [0.9, 8.8]	5.78	0.018
ANKK1 TaqIA	51.5 ± 1.6	49.3 ± 1.4	2.2 [−1.7, 6.0]	1.26	0.264
Gender (male)	–	–	−4.0 [−7.8, −1.1]	4.13	0.044
Post-traumatic amnesia (+)	–	–	−2.6 [−6.7, 1.6]	1.51	0.221
ED admission GCS (per unit)	–	–	−0.1 [−0.8, 0.5]	0.15	0.699
CT intracranial pathology (+)	–	–	−5.8 [−10.0, −1.6]	7.46	0.007
CVLT-II Short-Delay Free Recall					
DRD2 C957T	10.9 ± 0.4	9.6 ± 0.5	1.3 [0.1, 2.5]	4.06	0.046
ANKK1 TaqIA	10.6 ± 0.5	9.9 ± 0.4	0.7 [−0.5, 1.9]	1.40	0.239
Gender (male)	–	–	−0.8 [−2.0, 0.4]	1.67	0.198
Post-traumatic amnesia (+)	–	–	−0.5 [−1.8, 0.8]	0.64	0.426
ED admission GCS (per unit)	–	–	0.0 [−0.2, 0.2]	0.18	0.676
CT intracranial pathology (+)	–	–	−1.7 [−3.0, −0.4]	6.74	0.011
CVLT-II Long-Delay Free Recall					
DRD2 C957T	11.5 ± 0.4	10.2 ± 0.5	1.3 [0.1, 2.5]	4.29	0.041
ANKK1 TaqIA	11.3 ± 0.5	10.5 ± 0.5	0.8 [−0.3, 2.0]	1.97	0.163
Gender (male)	–	–	−0.8 [−2.0, 0.4]	1.70	0.195
Post-traumatic amnesia (+)	–	–	−0.2 [−1.4, 1.1]	0.05	0.817
ED admission GCS (per unit)	–	–	0.1 [−0.1, 0.2]	0.24	0.622
CT intracranial pathology (+)	–	–	−2.0 [−3.3, −0.7]	9.70	0.002

Distributions are reported as mean ± standard error of the raw score for each cognitive measure by *DRD2 C957T* and *ANKK1 TaqIA* genotypes, adjusted for age and education years. The mean difference (*B*) is presented as the increase or decrease of the denoted category from the reference category for gender (male vs. female), post-traumatic amnesia (positive vs. negative), admission GCS (per-unit increase), and CT intracranial pathology (positive vs. negative). Higher scores suggest improved performance

CT computed tomography, *CVLT* California Verbal Learning Test, Second Edition, *ED* emergency department, *GCS* Glasgow Coma Scale

GCS did not show significant associations with CVLT-II Trials 1–5.

On multivariable analysis of CVLT-II Short-Delay Free Recall, the *DRD2* T-allele showed a significant association with improved performance (mean increase 1.2 points, 95% CI [0.1 to 2.5], $p = 0.046$). CT pathology was the only other significant multivariable predictor (mean decrease 1.7 points, 95% CI [−3.0 to −0.4], $p = 0.011$) (Table 3).

On multivariable analysis of CVLT-II Long-Delay Free Recall, the *DRD2* T-allele showed a significant association with improved performance (mean increase 1.3 points, 95% CI [0.1 to 2.5], $p = 0.041$). CT pathology was the only other significant predictor (mean decrease 2.0 points, 95% CI [−3.3 to −0.7], $p = 0.002$) (Table 3).

DRD2 C957T is not associated with processing speed or mental flexibility after multivariable correction

As previously demonstrated (Table 2), no significant differences were observed between the *DRD2* C957T polymorphism and TMT B-A or WAIS-PSI. To confirm the lack of confounder effects, we utilized a similar multivariable approach for TMT B-A and WAIS-PSI (Table 4). On multivariable analysis, a non-significant statistical trend was observed for *DRD2 C957T* T-carriers on TMT B-A (mean decrease −16.2 s, 95% CI [−34.6 to 2.2], $p = 0.084$), while no other predictors showed a significant association. No significant association was observed on WAIS-PSI for *DRD2* T-allele carriers (mean increase 1.1 points, 95% CI [−4.6 to 6.9], $p = 0.700$) or any other predictor, and only admission GCS showed a non-significant statistical trend (per-unit increase of 0.8 points, 95% CI [−0.1 to 1.7, $p = 0.093$). These data confirm that the *DRD2 C957T* polymorphism does not associate with 6-month performance on metrics of non-verbal processing speed or mental flexibility.

Discussion

In the present study, we investigated whether the *DRD2 C957T* polymorphism was associated with cognitive performance 6 months following TBI. We show that the *DRD2 C957T* polymorphism was associated with better performance on the

Table 4 Multivariable analysis of 6-month mental flexibility and non-verbal processing speed performance by *DRD2 C957T* genotype

Predictor	T-Present	T-Absent	*B* [95% CI]	*F*-ratio	Sig. (*p*)
TMT Trail B minus A					
DRD2 C957T	48.8 ± 6.0	65.0 ± 7.9	−16.2 [−34.6, 2.2]	3.03	0.084
ANKK1 TaqIA	51.8 ± 7.2	62.0 ± 6.5	−10.2 [−27.8, 7.5]	1.30	0.257
Gender (male)	–	–	−8.8 [−26.6, 9.0]	0.96	0.329
Post-traumatic amnesia (+)	–	–	−5.9 [−25.0, 13.3]	0.37	0.545
ED admission GCS (per unit)	–	–	0.5 [−2.5, 3.5]	0.12	0.733
CT intracranial pathology (+)	–	–	−7.7 [−27.0, 11.5]	0.63	0.429
WAIS-PSI composite score					
DRD2 C957T	100.4 ± 1.9	99.3 ± 2.4	1.1 [−4.6, 6.9]	0.15	0.700
ANKK1 TaqIA	99.8 ± 2.2	100.0 ± 2.0	−0.2 [−5.8, 5.3]	0.01	0.931
Gender (male)	–	–	0.7 [−4.8, 6.3]	0.07	0.792
Post-traumatic amnesia (+)	–	–	0.0 [−6.0, 5.9]	0.00	0.997
ED Admission GCS (per unit)	–	–	0.8 [−0.1, 1.7]	2.87	0.093
CT intracranial pathology (+)	–	–	0.9 [−5.0, 6.8]	0.10	0.758

Distributions are reported as mean ± standard error of the raw score for each cognitive measure by *DRD2 C957T* and *ANKK1 TaqIA* genotypes, adjusted for age and education years for TMT Trail B minus Trail A and adjusted for education years for WAIS-PSI as it is already normed for age. The mean difference (B) is presented as the increase or decrease of the denoted category from the reference category for gender (male vs. female), post-traumatic amnesia (positive vs. negative), admission GCS (per-unit increase), and CT intracranial pathology (positive vs. negative). Lower scores on TMT suggest improved performance. Higher scores on WAIS-PSI suggest improved performance

CT computed tomography, *ED* emergency department, *GCS* Glasgow Coma Scale, *TMT* Trail Making Test, *WAIS-PSI* Wechsler Adult Intelligence Scale, Fourth Edition Processing Speed Index

components of the CVLT but was not the WAIS-PSI or the TMT. The CVLT assesses a patient's ability to store new information and is understood to be a gauge of verbal and working memory [33, 39]. Thus, our results suggest that the *DRD2 C957T* polymorphism is *specifically* associated with better verbal and working memory post-TBI and does not offer benefit for processing speed and/or mental flexibility. The identification of a potential association with *DRD2* and cognitive outcome after TBI and the specificity of the effect for verbal and working memory are both novel insights advanced by this work.

Previous efforts to associate *DRD2* with altered cognitive performance have been promising but inconclusive. In 2005, a study found an association between *DRD2* SNPs and altered cognitive performance in a post-TBI population [18]. However, these results were confounded by the influence of a nearby gene, *ANKK1* [13]. It was not known if *DRD2* is independently associated with long-term altered cognitive performance in a post-TBI population. Here, we analyzed subjects' cognitive performance at 6 months after TBI and controlled for the effects of *ANKK1*. The 6-month time point allowed us to measure long-term cognitive outcome after TBI and not be overly influenced by transient changes in cognition that occur during the recovery period, which usually completes 3 months after injury [40, 41]. As noted previously, we found that *DRD2* genotype was associated with cognitive differences at 6 months when the *ANKK1* effects were included in the multivariate regression. Thus, these data support the idea that *DRD2 C957T* may be an independent predictor of cognitive outcome after TBI.

A recent study by Failla et al. conducted in 108 severe TBI patients investigating rs6279, a gene with considerable linkage disequilibrium with rs6277, suggests that differences attributable to the *DRD2 C957T* polymorphism may not be maintained at 12 months [42]. Our findings showing an advantage of *DRD2 C957T* at 6 months raise questions as to whether C957T carriers may endure an altered trajectory of recovery and experience delayed recovery sometime within the 6–12-month interval. It also may suggest that the cognitive deficits are not altogether permanent. An alternative explanation is that the severity of the injury could interact with cognitive recovery. Specifically, the work by Failla et al. focused on severe TBI subjects of all races with positive intracranial pathology on CT that received treatment from a level I trauma center, whereas our data included data from all TBI patients of Caucasian race, with a mixture of CT pathology [42]. The resolving deficit in Failla et al. could be due to the extensive treatments that this cohort offered and may not be generalizable to all TBI patients [42].

Establishing that the *DRD2* polymorphism is associated with cognitive outcome after TBI also may explain the variability in response to dopamine therapy after TBI. Indeed, there have been six randomized controlled trials examining the role of amantadine and/or bromocriptine (both dopamine-enhancing agents) in cognitive recovery after TBI; the results have not shown a consistent benefit of dopamine agents in

cognitive recovery and are often discordant [43]. However, here, we show that the presence of *DRD2* polymorphisms may influence cognitive recovery after TBI, and it is very likely that the effect of dopamine agents will be heavily influenced on their presence as well as those of related polymorphisms in dopaminergic catabolic biochemical pathways—such as catechol-o-methyltransferase. Thus, we recommend that future studies examining dopamine agents as a treatment for TBI stratify patients based on the presence of the *DRD2* genotype, which may clarify the role of dopamine therapy in TBI.

Aside from establishing a potential association between *DRD2* and cognitive outcome after TBI, we also show that *DRD2 C957T* may specifically associate with improved verbal and working memory. This specificity is important because it shows that *DRD2* genotype likely does not enhance global cognitive ability, such as attention and awareness, which may covary with many different cognitive outcomes. Instead, there may be a specific link between *DRD2* and verbal and working memory. This link can be explained by the fact that the D2 dopamine receptor has enriched expression in the basal ganglia, a region important in learning and memory [44]. Furthermore, dopaminergic neurons in the basal ganglia (substantia nigra pars compacta) project directly to the prefrontal cortex and the hippocampus, regions that have been heavily implicated in working and verbal memory, respectively [45].

Limitations

Our results provide a link between the genetic, neuroscience, and psychological markers of cognitive dysfunction after TBI. However, there are a number of caveats that should be mentioned. First, although it has been speculated that patients' genotypes can alter the magnitude dopamine expression and dopamine binding, which could change the course of their recovery [46], other studies have shown that *C957T* is associated with increased risk for some neuropsychiatric diseases [25, 47]. Therefore, it is not clear if different *DRD2* genotypes confer a baseline difference in CVLT performance or if they signify altered performance after TBI. Second, our sample consisted exclusively of Caucasian patients, and consequently, our findings may not generalize to the population as a whole. Third, we only considered patients' GCS score when producing our multivariable models. These models, therefore, did not factor in possible disparate courses of prior medications, post-injury medical treatment, or rehabilitative therapy. Furthermore, we were limited by a relatively small sample size of 128 patients without controls. While the NINDS CDE outcome domains are generally distinct, the possible overlap across cognitive symptomatologies attributable to *DRD2 C957T* will benefit from a rigorous case-control study adequately powered to adjust for a range of comparisons. We were also constrained to specifications of the NINDS CDE version 1, which were limited to 6 months post-injury; as cognitive deficits following TBI may change with time after injury, an analysis tracking the trajectory of recovery for *DRD2 C957T* variants constitutes an important future direction. Lastly, the true effect of *DRD2* variants is difficult to establish due to presumed gene-gene interactions. The genetic variation in *DRD2* genes may interact with effects induced by other genes important for cognitive recovery.

Conclusions

The *DRD2 C957T* polymorphism (rs6277) is associated with verbal memory performance at 6 months following TBI independent of the *ANKK1 TaqIA* polymorphism (rs1800497), while no associations were seen on measures of non-verbal processing speed or mental flexibility, in a sample of Caucasian patients. Larger studies in more diverse populations will be necessary to confirm the influence of *DRD2 C957T* in these and other outcome domains following TBI. Whether a subgroup of patients with the *DRD2 C957T* polymorphism may benefit from closer clinical surveillance or targeted dopaminergic therapies remains to be determined and constitutes an important direction for future research.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Appendix

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Original Articles

Diffusion Tensor Imaging for Outcome Prediction in Mild Traumatic Brain Injury: A TRACK-TBI Study

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Abstract

We evaluated 3T diffusion tensor imaging (DTI) for white matter injury in 76 adult mild traumatic brain injury (mTBI) patients at the semiacute stage (11.2 ± 3.3 days), employing both whole-brain voxel-wise and region-of-interest (ROI) approaches. The subgroup of 32 patients with any traumatic intracranial lesion on either day-of-injury computed tomography (CT) or semiacute magnetic resonance imaging (MRI) demonstrated reduced fractional anisotropy (FA) in numerous white matter tracts, compared to 50 control subjects. In contrast, 44 CT/MRI-negative mTBI patients demonstrated no significant difference in any DTI parameter, compared to controls. To determine the clinical relevance of DTI, we evaluated correlations between 3- and 6-month outcome and imaging, demographic/socioeconomic, and clinical predictors. Statistically significant univariable predictors of 3-month Glasgow Outcome Scale-Extended (GOS-E) included MRI evidence for contusion (odds ratio [OR] 4.9 per unit decrease in GOS-E; $p = 0.01$), ≥1 ROI with severely reduced FA (OR, 3.9; $p = 0.005$), neuropsychiatric history (OR, 3.3; $p = 0.02$), age (OR, 1.07/year; $p = 0.002$), and years of education (OR, 0.79/year; $p = 0.01$). Significant predictors of 6-month GOS-E included ≥1 ROI with severely reduced FA (OR, 2.7; $p = 0.048$), neuropsychiatric history (OR, 3.7; $p = 0.01$), and years of education (OR, 0.82/year; $p = 0.03$). For the subset of 37 patients lacking neuropsychiatric and substance abuse history, MRI surpassed all other predictors for both 3- and 6-month outcome prediction. This is the first study to compare DTI in individual mTBI patients to conventional imaging, clinical, and demographic/socioeconomic characteristics for outcome prediction. DTI demonstrated utility in an inclusive group of patients with heterogeneous backgrounds, as well as in a subset of patients without neuropsychiatric or substance abuse history.

Key words: axonal injury; computed tomography; diffusion tensor imaging; magnetic resonance imaging; traumatic brain injury

Introduction

Mild traumatic brain injury (mTBI) comprises 75% of the estimated 1.7 million patients who seek medical attention annually in the United States for acute head injury.[1] The most widely accepted definitions of mTBI[2–4] include patients with 1) non-penetrating head trauma resulting in one or more of the following: confusion/disorientation; loss of consciousness (LOC) <30 min in duration, post-traumatic amnesia (PTA) <24 h in duration; and transient focal neurological signs or seizure and 2) Glasgow Coma

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Scale (GCS) score of 13–15 upon acute medical evaluation. Previous studies suggest that many mTBI patients have significant alterations in cognitive and/or behavioral functioning within weeks to months of injury, and approximately 15–20% have persistent measurable deficits at 1 year.[5–12] There is also growing recognition that current classification schemes for mTBI/concussion based solely on GCS, PTA, and LOC are severely limited, with small *mean* effect sizes in long-term impairment obscuring differences among diverse subgroups of mTBI patients with very different prognoses.[13,14] To date, there remains a need for practical, widely available clinical, laboratory, and/or imaging markers that identify patients who will experience persistent dysfunction after mTBI.

Many studies have reported changes in white matter diffusion tensor imaging (DTI) parameters in acute, subacute, and chronic time frames after mTBI.[15–37] The clinical significance of acute traumatic intracranial findings on conventional computed tomography (CT) and magnetic resonance neuroimaging has also been explored.[38,39] However, little is known about the relationship between conventional CT and magnetic resonance imaging (MRI) findings and DTI evidence of white matter injury within the mTBI spectrum. In addition, there has been little exploration of the use of acute or subacute DTI data for prediction of outcome in individual patients, after controlling for demographic, clinical, and CT and conventional MRI predictors. Although group differences in DTI parameters between mTBI patients and controls have been demonstrated, no consensus yet exists on the practical application of these techniques to outcome prediction in the individual patient. Finally, nearly all previous studies of DTI in mTBI have excluded patients with any history of substance abuse or other neuropsychiatric disorder, and the generalizability of their results to the general mTBI population is uncertain.

In this study, we used both whole-brain voxel-wise and region-of-interest (ROI) analyses to assess for an association between CT and conventional MRI abnormalities and early DTI measures of white matter integrity after mTBI. To determine the clinical relevance, if any, of DTI measures to outcome in mTBI, we then assessed for correlations between DTI measures and 3- and 6-month outcome. We compared the strengths of these correlations to those between outcome and conventional imaging, demographic, and clinical predictors previously found to influence outcome, based on the assumption that any utility of DTI in outcome prediction would require a *differential* increase in predictive power over predictors that are routinely assessed in current practice. To our knowledge, this is the first study to compare the relative strengths of DTI features in individual mTBI patients to conventional MRI, CT, clinical, demographic, and socioeconomic features for the prediction of 3- and 6-month outcome. In order to maximize the generalizability of study conclusions, we analyzed both an inclusive sample of 76 mTBI patients with very few exclusion criteria, as well as a subset of 37 patients with no significant drug, alcohol, or neuropsychiatric history.

Methods

Study population

mTBI patients were enrolled at San Francisco General Hospital (SFGH; San Francisco, CA) as part of the prospective multi-center TRACK-TBI (Transforming Research and Clinical Knowledge in Traumatic Brain Injury) pilot study.[40] The primary inclusion criterion for the TRACK-TBI pilot study was performance of non-contrast head CT to assess for evidence of acute TBI within 24 h of injury, based on criteria from the American College of Emergency Physicians/Centers for Disease Control (ACEP/CDC) evidence-based joint practice guideline (Supplementary Table S1) (see online supplementary material at http://www.liebertpub.com).[41] The TRACK-TBI pilot study exclusion criteria were limited and consisted of nonfluency in English, contraindication to MRI, pregnancy, and current incarceration/legal detention or placement on psychiatric hold.[40]

For the current study of DTI of mTBI, additional inclusion criteria were GCS 13–15 upon emergency department (ED) arrival, LOC <30 min, PTA duration <24 h, and age 18–55 years (inclusive); an additional exclusion criterion was any reported history of earlier TBI resulting in LOC >5 min. Of 190 mTBI patients in the 18- to 55-year age range enrolled at SFGH for the TRACK-TBI pilot study, 87 patients did not undergo brain MRI. Of the remaining 103 patients, 18 reported a history of earlier TBI with LOC >5 min or of unknown duration; 5 had a technically inadequate brain MRI exam (because of motion or, in 1 case, because of severe susceptibility artifact resulting from a metallic shunt valve within the scalp); 1 patient had an extensive area of encephalomalacia likely the result of an earlier TBI; 1 had an acute large-territory infarct resulting from acute traumatic arterial dissection; and 2 were excluded because their performance on the Trail Making Test (TMT) B and other outcome measures were extreme outliers, despite a GCS of 15 upon ED arrival, no LOC or PTA, and no CT or conventional MRI evidence of traumatic intracranial injury. The final patient group for the current study therefore consisted of 76 mTBI patients enrolled at SFGH who underwent brain MRI on a single 3T MRI scanner within 3 weeks of TBI. In addition, a control group consisted of 50 healthy subjects, ages 18–55 years, with no self-reported history of drug or alcohol abuse, neuropsychiatric illness, or earlier TBI, who underwent brain MRI on the same 3T scanner over the same time period, employing the same MRI protocol and software version. All study protocols were approved by the University of California at San Francisco Institutional Review Board, and all patients and control subjects or their legal representatives gave written informed consent.

Table 1 summarizes demographic, socioeconomic, and clinical characteristics of participants and control subjects. We assessed for statistically significant differences in demographic, socioeconomic, and clinical features at $p<0.05$ among the following groups: 1) CT/MRI-positive patients, defined as patients with any acute traumatic intracranial lesion or depressed skull fracture on day-of-admission CT or semiacute 3T MRI; 2) CT/MRI-negative patients, defined as patients without any such abnormality; and 3) control subjects. We used analysis of variance (ANOVA) for scale variables without significant deviation from a normal distribution, and Mann-Whitney U test for ordinal and non-normal variables. Differences in nominal variables were assessed by chi-square (χ^2) test for independence or by Fisher's exact test for nominal variables with an expected count of fewer than 5 subjects in any cell. All statistical analyses were performed using SPSS Statistics (version 21; SPSS, Inc., Chicago, IL).

CT and MRI protocols

CT was performed within 2 h 42 min ± 3 h 9 min of TBI. MRI was performed within 11.2 ± 3.3 days (range, 5–18) postinjury. All CT exams were performed on a GE Lightspeed 64-row-detector CT scanner, and all MRI exams were performed on the same 3T GE Signa EXCITE scanner equipped with an eight-channel phased-array head radiofrequency coil (GE Healthcare, Waukesha, WI), using the same scanner software version. Whole-brain DTI was performed with a multi-slice single-shot spin echo echoplanar pulse sequence (echo time [TE] = 63 ms; repetition time [TR] = 14 sec) using 55 diffusion-encoding directions, isotropically distributed over the surface of a sphere with electrostatic repulsion, acquired at $b=1000$ sec/mm^2, seven acquisitions at $b=0$ sec/mm^2, 72 interleaved slices of 1.8-mm thickness each with no gap between slices, a 128×128 matrix, and a field of view (FOV) of 230×230 mm.

Table 1. Demographic, Socioeconomic, and Clinical Predictors for 76 mTBI Patients and 50 Control Subjects

Predictors	*CT/MRI-negative mTBI (no acute traumatic intracranial abnormality or depressed skull fracture on CT and/or conventional MRI) (44 subjects)*	*CT/MRI-positive mTBI (acute traumatic intracranial abnormality and/or depressed skull fracture on CT and/or conventional MRI) (32 subjects)*	*Controls (50 subjects)*	*Analysis for group differences among CT/MRI-negative mTBI, CT/MRI-positive mTBI, and control subjects*	
Demographic and socioeconomic					
Age (years, mean ± standard deviation)	31.2 ± 9.5	33.9 ± 12.0	28.7 ± 9.2	F (2,123) = 2.6; $p = 0.08$	ANOVA
Education (years, mean ± standard deviation)	14.8 ± 2.8	14.6 ± 2.1	15.7 ± 1.6	F (2,109) = 2.6; $p = 0.08$	
Gender: male/female (% male)	27/17 (61%)	23/9 (72%)	32/18 (64%)	χ^2 (2; $n = 126$) = 0.9; $p = 0.65$	χ^2 test for independence
Unemployed[a] : yes/no (% yes)	5/39 (11%)	6/25 (19%)	Unknown	$p = 0.51$	Fisher's exact test
Handedness[b] (right/left/ambidextrous)	39/4/1	27/4/1	48/1/0	$p = 0.14$	
Clinical					
Neuropsychiatric history: yes/no (% yes)	12/32 (27%)[d]	6/26 (19%)[d]	0/50 (0%)[e]	χ^2 (2; $n = 126$) = 14.9; $p = 0.0004$	χ^2 test for independence
History of drug or alcohol problem: yes/no (% yes)	21/23 (48%)[f]	14/18 (44%)[f]	0/50 (0%)[g]	χ^2 (2; $n = 126$) = 32.0; $p < 10^{-6}$	
LOC: yes, up to 30 min/no (% yes)	28/16 (64%)	23/9 (72%)	N/A	χ^2 (1; $n = 76$) = 0.6; $p = 0.47$	
PTA: yes/no (% yes)	26/18 (59%)	25/7 (78%)	N/A	χ^2 (1; $n = 76$) = 3.0; $p = 0.09$	
PTA duration[c]	None 18 < 1 min 6 1–29 min 14 30–59 min 3 1–24 h 3	None 7 < 1 min 1 1–29 min 11 30–59 min 5 1–24 h 4	N/A	CT/MRI-negative median PTA duration < 1 min; CT/MRI-positive median PTA duration 1–29 min; U = 440; z = −2.1; $p = 0.03$	Mann-Whitney U test
GCS (15/14/13)	36/7/1	20/11/1	N/A	$p = 0.13$	Fisher's exact test
Previous TBI with LOC up to 5 min: yes/no (% yes)	15/29 (34%)[h]	8/24 (25%)[h]	0/50 (0%)[i]	$p = 0.000003$	

Gray shaded boxes indicate statistically significant difference at $p < 0.05$.
[a]One CT/MRI-positive mTBI patient with unknown employment status was not included in this analysis.
[b]One control with unknown handedness was not included in this analysis.
[c]Four CT/MRI-positive mTBI patients with PTA < 24 h, but not otherwise specified, were not included in this analysis.
[d–i]Each superscript denotes a subset of participants whose proportions do not significantly differ from each other at $p < 0.05$ by Pearson's χ^2 test (or Fisher's exact test when expected cell count < 5).
mTBI, mild traumatic brain injury; ANOVA, analysis of variance; GCS, Glasgow Coma Scale; PTA, post-traumatic amnesia; LOC, loss of consciousness; N/A, not available.

Parallel imaging was employed using the array spatial sensitivity encoding technique (ASSET) with an acceleration factor of 2.

The following conventional 3T MRI sequences were also performed: 1) axial three-dimensional (3D) inversion recovery fast spoiled gradient recalled echo T1-weighted images (TE = 1.5 ms; TR = 6.3 ms; inversion time [TI] = 400 ms; flip angle, 15 degrees) with 230-mm FOV, 156 contiguous partitions (1.0-mm) at 256 × 256 matrix; 2) axial T2-weighted fluid-attenuated inversion recovery images (TE = 126 ms; TR = 10 sec; TI = 2200 ms) with 220 mm FOV, 47–48 contiguous slices (3.0-mm) at 256 × 256 matrix; and 3) axial magnetization-prepared gradient echo T2*-weighted images (TE = 15 ms; TR = 500 ms; flip angle 20 degrees) with 220 × 170 mm FOV and 47–48 contiguous slices (3.0-mm) at 256 × 192 matrix.

Neuroradiologist evaluation of CT and MRI studies for acute traumatic abnormalities

Each patient's head CT upon ED presentation and early brain MRI (11.2 ± 3.3 days postinjury) was characterized using the TBI common data elements (TBI-CDE). The TBI-CDEs are consensus-based recommendations for data collection, data definitions, and best practices in TBI research established jointly by the National Institute of Neurological Disorders and Stroke (NINDS), Defense Centers of Excellence, National Institute on Disability and Rehabilitation Research, and Veterans Administration.[42–44] Each CT and MRI was anonymized and reviewed by a board-certified neuroradiologist blinded to demographic, socioeconomic, and clinical data, except gender and age, and without concurrent access to the patient's other head imaging studies or 3- and 6-month outcome measures.

mTBI patients were divided into two subgroups: 1) CT/MRI positive, defined as patients with any acute traumatic intracranial lesion (epidural hematoma [EDH], subdural hematoma [SDH], subarachnoid hemorrhage [SAH], contusion, or evidence of traumatic axonal injury) and/or depressed skull fracture on either CT or MRI, and 2) CT/MRI negative, defined as patients without any such abnormality. Most previous studies of ''complicated'' mTBI, including Williams and colleagues,[38] demonstrated poorer neuropsychiatric test performance based solely on CT findings (presence of any acute intracranial hemorrhage or depressed skull fracture). Our dichotomization of mTBI patients according to presence of abnormalities on *either* CT or MRI is based on more recent work that demonstrated poorer 3-month outcome associated with early MRI intracranial abnormalities, whether or not visible on CT.[39]

Diffusion tensor image processing

Nonbrain tissue was eliminated from the diffusion-weighted and 3D T1-weighted images using the Functional MRI of the Brain (FMRIB, Oxford University, Oxford, UK) Brain Extraction Tool.[45] Diffusion-weighted images were corrected for eddy currents and registered to the $b = 0\ sec/mm^2$ volume using the FMRIB Linear Image Registration Tool. A diffusion tensor model was constructed using the FMRIB DTIFit algorithm[46] to yield fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) at each voxel. Tract-based spatial statistics (TBSS)[47] were used to align each subject's FA data to a white matter skeleton, after low FA values below a threshold of 0.25 were excluded to limit voxels to the white matter.

Voxel-wise nonparametric statistical comparison between 76 mTBI patients and 50 controls was performed using the FMRIB Software Library (FSL) randomise algorithm based on permutation testing, with corrections for multiple voxel-wise comparisons using threshold-free cluster enhancement (TFCE).[48] Anatomic locations of voxel clusters with statistically significant differences in FA, MD, RD, or AD between mTBI and control groups at $p < 0.05$ were determined. This analysis was also used to compare the subgroup of 32 CT/MRI-positive patients to the 50 controls and also the subgroup of 44 CT/MRI-negative mTBI patients to the 50 controls.

In addition to the whole-brain voxel-wise approach, we performed a complementary ROI analysis to address the possibility that a whole-brain, data-driven approach might not be sufficiently sensitive to reveal white matter injury because of possibly significant spatial heterogeneity of white matter injury across mTBI subjects. Twenty-seven white matter ROIs were delineated by the intersection of the Johns Hopkins University (Baltimore, MD) ICBM-DTI-81 White Matter Labeled Atlas[49] and the reference white matter skeleton. These consisted of the anterior corona radiata, superior corona radiata, posterior corona radiata, anterior limb of internal capsule, posterior limb of internal capsule, external capsule, superior longitudinal fasciculus, sagittal striatum, ventral cingulum (parahippocampal gyrus), dorsal cingulum (cingulate gyrus), inferior fronto-occipital fasciculus, and superior fronto-occipital fasciculus, each on the left and right; and also the body, genu, and splenium of the corpus callosum. The FA, MD, AD, and RD within each of these 27 ROIs in each patient and control subject were determined. For each ROI, the mean and standard deviation (SD) of the FA within the group of 50 control subjects was calculated. Similarly, for each ROI, the mean and SD for each of the other DTI measures (MD, AD, and RD) in the group of 50 control subjects were calculated. For each of the 76 mTBI patients and 50 control subjects, an abnormal ROI was then defined as one in which a DTI measure (FA, MD, AD, or RD) was more than 2.2 SDs below or above the control-group mean, based on the distribution of the DTI measure within the 50 control patients alone.

Outcome measures

Outcome measures included the Extended Glasgow Outcome Scale (GOS-E) at 3 and 6 months postinjury, the Rivermead Postconcussion Symptoms Questionnaire (RPQ), California Verbal Learning Test–Second Edition (CVLT-II), Wechsler Adult Intelligence Scale–Fourth Edition, Processing Speed Index (WAIS-IV PSI), and Trail Making Tests A and B (TMT A and TMT B) at 6 months. The GOS-E was obtained at 3 and 6 months postinjury through structured interview with each participant by research assistants trained to uniformly assess the GOS-E. Modeled after the 5-point Glasgow Outcome Scale (GOS), the 8-point GOS-E provides better discrimination among more subtle aspects of disability within mild-to-moderate, rather than mild-to-severe, TBI and is a well-validated, widely employed measure of global function after mTBI.[50] The TMT A and B are tests of visual attention, visual-motor coordination, task switching, and executive function.[51,52] WAIS-IV PSI is a test of perceptual processing speed with additional contribution from working memory.[53,54] The CVLT-II is a test of verbal learning and memory and was used in place of the TBI CDE Rey Auditory Verbal Learning Test because of recent revision of the CVLT with demonstration of improved psychometric properties.[55,56] The RPQ consists of 16 symptoms frequently reported after mTBI.[57,58] The first three symptoms, denoted RPQ-3, are more physical symptoms (headaches, dizziness, and nausea/vomiting) typically experienced immediately after the TBI event, whereas the other 13 symptoms (denoted RPQ-13) are more psychosocial in nature (hyperacusis, sleep disturbances, fatigue, irritability, depressed mood, frustration, forgetfulness, poor concentration, requiring longer times to think, blurred vision, light sensitivity, double vision, and restlessness) and have been shown to occur later in the clinical course after mTBI.[59,60]

We assessed for statistically significant group differences in each outcome measure between CT/MRI-positive and -negative mTBI patients. The CVLT-II, WAIS-IV PSI, and TMT A and B scores were converted to normative scores for age, and ANOVA was used to test for group differences in these variables between CT/MRI-positive and -negative mTBI patients at $p < 0.05$. Mann-

Whitney U test was used to assess for group differences in the 3-month GOS-E, 6-month GOS-E, RPQ-3, and RPQ-13 at $p < 0.05$. All statistical analyses were performed using SPSS Statistics (version 21).

Spearman's correlation and ordinal logistic regression analyses

We calculated Spearman's correlation coefficients between each outcome measure and each of 11 demographic (age, gender), socioeconomic (employment status, number of years of formal education), and clinical (history of major neuropsychiatric diagnosis, history of drug or alcohol abuse, GCS upon ED arrival, any PTA, PTA duration, any LOC, any history of mTBI with LOC duration not exceeding 5 min) predictors, 5 noncontrast head CT features (calvarial or skull base fracture, EDH, SDH, SAH, contusion), and 3 brain MRI features (contusion, hemorrhagic axonal injury, or evidence of white matter injury on DTI ROI analysis). We used Spearman's correlation, rather than its parametric counterpart, Pearson's product-moment correlation, because of the nominal or ordinal nature and/or non-normal distribution of most of these variables. We then performed multivariable logistic or linear regression of each outcome measure upon all predictors with which the outcome measure had demonstrated a statistically significant pairwise Spearman's correlation. For both Spearman's correlation and the regression analyses, the CVLT-II, WAIS-IV PSI, and TMT A and B test scaled or z-scores, as well as binary outcome variables corresponding to performance worse or better than 2 SDs worse than the normative score as determined by previous studies,[52,54,55] were included as outcome variables. For the ordinal logistic regression analyses, tests for parallel lines were performed and confirmed the proportional odds assumption for each analysis. These statistical analyses were performed using SPSS Statistics (version 21).

Results

Study population characteristics

Table 1 summarizes demographic, socioeconomic, and clinical characteristics of participants. There were no statistically significant differences among CT/MRI-positive, CT/MRI-negative, and control subjects in age, number of years of formal education, gender, or handedness. Employment status was unknown for control subjects, but there was no difference at $p < 0.05$ between CT/MRI-positive and -negative patients. Among the clinical variables, rates of major neuropsychiatric diagnosis, history of drug or alcohol abuse, and history of previous mTBI with LOC up to 5 min were significantly higher in CT/MRI-negative and -positive mTBI patients than in control subjects, but were not statistically different between CT/MRI-negative and -positive patients. (Patients with a history of any previous TBI with LOC > 5 min had been excluded from the study.) PTA duration was longer in CT/MRI-positive patients (median PTA duration, 1–29 min) than in CT/MRI-negative patients (median PTA duration, < 1 min). There was no significant difference in GCS or LOC between CT/MRI-negative and -positive mTBI groups at $p < 0.05$ (Table 1).

Conventional CT and MRI results

Table 2 shows that MRI identifies many more acute traumatic intracranial lesions than CT. TBI-CDE–defined pathoanatomic features observed on head CT upon ED presentation and early brain MRI in our study population consisted of the following: nondepressed skull fracture; EDH; SDH; SAH; brain contusion; and hemorrhagic axonal injury. Hemorrhagic axonal injury was observed on many brain MRI exams, but on only one head CT, in this study. Other TBI-CDE features, such as midline shift ≥ 5 mm and partial or complete basal cistern effacement that are more characteristic of moderate-to-severe TBI, were also not observed on any head CT or brain MRI in this study. In addition, no depressed skull fracture was observed in this study. As shown in Table 2, all 4 of 4 (100%) patients with CT evidence of contusion also had MRI evidence of contusion ± hemorrhagic axonal injury. In contrast, 7 of 11 (64%) patients with MRI evidence of contusion and 25 of 27 (93%) with MRI evidence of hemorrhagic axonal injury had no CT evidence of any parenchymal injury. Three patients with nondepressed skull fractures had no CT or conventional MRI traumatic intracranial abnormality and were classified as CT/MRI-negative mTBI (analogous to the classification of patients with isolated nondepressed skull fracture and no acute intracranial hemorrhage as ''uncomplicated'' mTBI in previous literature[38]).

Whole-brain voxel-wise nonparametric statistical comparison of diffusion tensor imaging measures in mTBI (n = 76) versus control subjects (n = 50)

Figure 1A shows many statistically significant areas of reduced FA in the 76 mTBI patients, compared to the 50 control subjects, using TBSS and voxel-wise nonparametric statistical comparison implemented in the FSL randomise algorithm and corrected for multiple comparisons with TFCE. mTBI patients demonstrated significantly lower FA in the right internal and external capsules,

Table 2. CT and Conventional MRI Findings in 76 mTBI Patients

	CT				
	Normal	*Nondepressed skull fracture only*	*Acute extraaxial hemorrhage (EDH, SDH, SAH) with no parenchymal injury*	*Contusion ± extraaxial hemorrhage*	*Hemorrhagic axonal injury only*
MRI					
No parenchymal injury	41	3	2	0	0
Hemorrhagic axonal injury only	17	0	1	0	1
Contusion only	0	0	0	3	0
Both hemorrhagic axonal injury and contusion	1	1	5	1	0

Gray shaded boxes comprise uncomplicated mTBI (no CT evidence of acute intracranial hemorrhage or depressed skull fracture).[38]

CT, computed tomography; MRI, magnetic resonance imaging; mTBI, mild traumatic brain injury; EDH, epidural hematoma; SDH, subdural hematoma; SAH, subarachnoid hemorrhage.

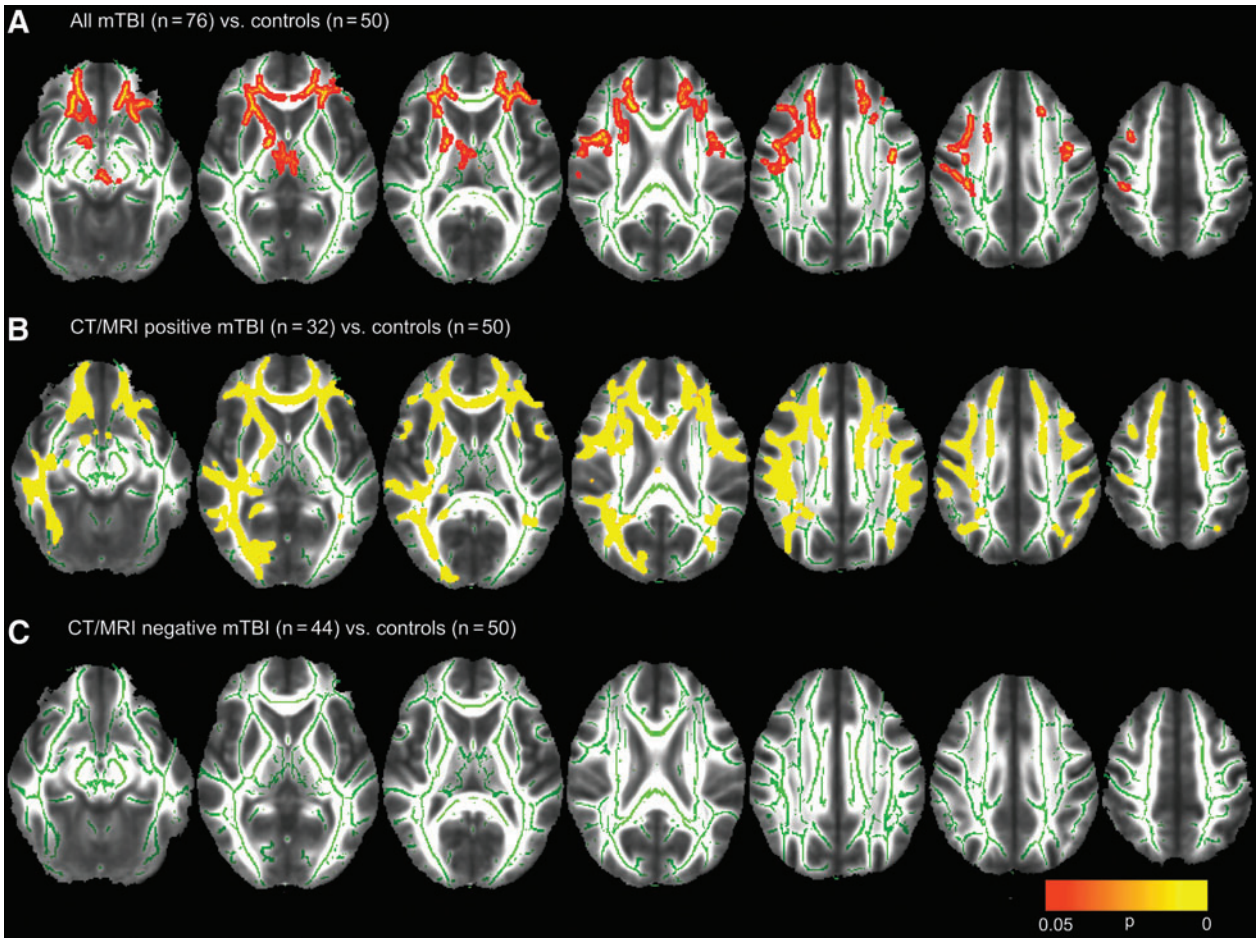

FIG. 1. Voxel-wise nonparametric statistical comparison between mild traumatic brain injury (mTBI) patients and controls, with corrections for multiple voxel-wise comparisons using threshold-free cluster enhancement. This analysis was used to compare (**A**) 76 mTBI patients to 50 controls, (**B**) the subgroup of 32 computed tomography/magnetic resonance imaging (CT/MRI)-positive mTBI patients to the 50 controls, and (**C**) the subgroup of 44 CT/MRI-negative patients to the 50 controls. Voxel clusters with statistically significant differences in fractional anisotropy (FA) between mTBI and control groups at $p < 0.05$ are shown in red/orange/yellow, with yellow denoting greater statistical significance. (A) shows that the 76 mTBI patients demonstrated significantly lower FA in the genu of the corpus callosum, uncinate fasciculi, and anterior corona radiata bilaterally as well as right internal and external capsules, compared to the 50 control subjects. (B) In a comparison of a much smaller subgroup of 32 CT/MRI-positive mTBI patients to the 50 controls, areas of reduced FA were even more extensive and attained much higher levels of statistical significance (yellow regions, corresponding to $p < 0.01$) than in the comparison of 76 mTBI patients to the control group (mostly red/orange areas, corresponding to $p < 0.05$, in [A]). (C) shows that this method demonstrated no evidence for white matter injury in 44 CT/MRI-negative mTBI patients, compared to the 50 controls. Color image is available online at www.liebertpub.com/neu

genu of the corpus callosum, and uncinate fasciculi and anterior corona radiata bilaterally.

No voxel with significantly increased FA, and no significant group differences in MD, RD or AD, were found in mTBI patients, compared to the control group at $p < 0.05$ using TBSS, randomise, and correction for multiple comparisons with TFCE.

Whole-brain voxel-wise nonparametric statistical comparison of diffusion tensor imaging measures in CT/MRI-positive mTBI (n = 32) versus control subjects (n = 50)

Figure 1B shows many highly statistically significant areas of reduced FA in the CT/MRI-positive subgroup of mTBI patients, compared to the control group. Despite the expected loss of statistical power for this comparison of a much smaller subgroup of 32 CT/MRI-positive mTBI patients to the control group, areas of reduced FA were even more extensive and attained higher levels of statistical significance (yellow regions, corresponding to $p < 0.01$; Fig. 1B) than in the comparison of 76 mTBI patients to the control group (mostly red/orange areas, corresponding to $p < 0.05$; Fig. 1A). mTBI patients demonstrated significantly lower FA in the genu and body of the corpus callosum, the external capsules, uncinate fasciculi, and anterior corona radiata bilaterally, the right internal capsule, and the right inferior longitudinal and inferior fronto-occipital fasciculi. Extensive areas of increased RD were also observed in the 32 CT/MRI-positive mTBI patients, relative to the control group, whereas none had been observed in the comparison of 76 mTBI patients to the control group. No voxel with increased FA or reduced RD was observed in CT/MRI-positive mTBI patients, relative to controls, at $p < 0.05$. There were also no voxels in which MD or AD differed significantly between CT/MRI-positive mTBI and control groups at $p < 0.05$.

Whole-brain voxel-wise nonparametric statistical comparison of diffusion tensor imaging measures in CT/MRI-negative mTBI (n = 44) versus control subjects (n = 50)

No significant group differences in FA (Fig. 1C), MD, RD, or AD were found between CT/MRI-negative mTBI and control groups at $p < 0.05$.

Whole-brain voxel-wise nonparametric statistical comparison of diffusion tensor imaging measures in most highly educated versus least educated control subjects (n = 50)

To exclude the possibility that the nonsignificant differences in educational level among CT/MRI-positive mTBI, CT/MRI-negative mTBI, and control groups (Table 1) could result in group differences in DTI parameters that could be erroneously attributed to mTBI, we assessed for group differences in DTI parameters between control subjects with the longest and shortest duration of education. The 50 control subjects were divided into two groups, one consisting of 25 patients with the most years of formal education and the other consisting of 25 patients with the fewest years of formal education. There were no statistically significant group differences in DTI parameters between these groups at $p < 0.05$. This analysis was performed to exclude the possibility that the statistically significant group differences in FA shown in Figure 1A and 1B were attributable mostly to educational level or to other socioeconomic factors that might be correlated with educational level.

Region-of-interest analysis of individual mTBI subjects

Table 3 shows that abnormally low FA (FA more than 2.2 SDs below the control-group mean) was observed in ≥ 1 ROIs for 14 of 32 CT/MRI-positive mTBI (43.8%), 11 of 44 CT/MRI-negative mTBI (25.0%), and 5 of 50 (10.0%) control subjects. Pearson's χ^2 test showed a highly significant difference between the proportions of CT/MRI-positive mTBI patients (43.8%) and control subjects (10.0%) with ≥ 1 abnormal ROIs ($p = 0.0006$). There was a trend toward a significant difference between the proportions of CT/MRI-negative mTBI patients (25.0%) and controls (10.0%) with ≥ 1 abnormal ROIs ($p = 0.06$). Finally, there was no significant difference between the proportions of CT/MRI-positive mTBI patients (43.8%) and CT/MRI-negative mTBI patients (25.0%) with ≥ 1 abnormal ROIs ($p = 0.14$).

Table 3 also shows that there was no significant difference ($p = 0.93$) among the proportions of CT/MRI-positive, CT/MRI-negative, and control subjects with ≥ 1 ROI with abnormally high FA (FA more than 2.2 SDs above the control-group mean).

Outcome measures

Table 4 summarizes 3- and 6-month outcome measures of participants. There were no statistically significant differences in any 3- or 6-month outcome measure between CT/MRI-negative and -positive mTBI groups at $p < 0.05$. For the TMT A and B, the actual times for test completion, the corresponding TMT A and B z-scores adjusted for age,[52] as well as the proportion of abnormal performances worse than 2 SDs from the age-adjusted mean, were compared between CT/MRI-positive and -negative mTBI groups, and none showed a statistically significant difference at $p < 0.05$.

Spearman's correlation

Table 5 shows the pair-wise Spearman's correlation coefficients between 3- and 6-month outcome measures and demographic, socioeconomic, clinical, CT, and MRI predictors. Gender, employment status, GCS at ED arrival, PTA, PTA duration, LOC, and history of previous TBI with LOC up to 5 min were not significantly correlated with any outcome variable, and these predictors were thus omitted from Table 5, for brevity. Similarly, worse outcomes, as measured by the 6-month TMT A (both age-adjusted z-score and the dichotomized score), TMT B (z-score), CVLT-II (both age-adjusted scaled score and dichotomized score), and WAIS-IV PSI

Table 3. DTI Region-of-Interest (ROI) Analysis: Group Differences in Presence of One or More Abnormal ROIs among CT/MRI-Negative mTBI, CT/MRI-Positive mTBI, and Control Subjects

	CT/MRI-negative mTBI (no acute traumatic intracranial abnormality or depressed skull fracture on CT or conventional MRI) (44 subjects)	*CT/MRI-positive mTBI (positive acute traumatic intracranial abnormality and/or depressed skull fracture on CT and/or conventional MRI) (32 subjects)*	*Controls (50 subjects)*
	Number of subjects (proportion of subjects)	*Number of subjects (proportion of subjects)*	*Number of subjects (proportion of subjects)*
One or more ROIs with FA more than 2.2 SDs below control-group mean	11 (25.0%)[a,b]	14 (43.8%)[b]	5 (10.0%)[a]
One or more ROIs with FA more than 2.2 SDs above control group mean	8 (18.2%)[c]	5 (15.6%)[c]	8 (16.0%)[c]

[a,b,c]Each superscript denotes a subset of participants whose column proportions do not differ significantly from one another, by Pearson's χ^2 test with $p < 0.05$. **Row 1:** There was a statistically significant difference between CT/MRI-positive mTBI (43.8%) and control subjects (10.0%), with one or more ROIs with FA more than 2.2 SDs *below* the control group mean ($p = 0.0006$). There was no significant difference between CT/MRI-negative mTBI patients (25.0%) and controls (10.0%; $p = 0.06$). There was also no significant difference between CT/MRI-positive (43.8%) and CT/MRI-negative mTBI patients (25.0%; $p = 0.14$). **Row 2:** There was no significant difference among the proportions of CT/MRI-negative mTBI (18.2%), CT/MRI-positive mTBI (15.6%), and control subjects (16.0%) with one or more ROIs with FA more than 2.2 SDs *above* the control group mean ($p = 0.96$).

DTI, diffusion tensor imaging; ROI, region of interest; CT, computed tomography; MRI, magnetic resonance imaging; mTBI, mild traumatic brain injury; FA, fractional anisotropy; SD, standard deviation.

Table 4. Group Differences in 3- and 6-Month Outcome Measures between 32 CT/MRI-Positive mTBI and 44 CT/MRI-Negative mTBI Patients

	CT/MRI-negative (no acute traumatic intracranial abnormality or depressed skull fracture on CT or conventional MRI) (44 subjects)		*CT/MRI-positive (acute traumatic intracranial abnormality or depressed skull fracture on CT and/or conventional MRI) (32 subjects)*		*Analysis for group differences between CT/MRI negative, CT/MRI positive*	
3-month outcome measure	*Score*	*Number of patients*	*Score*	*Number of patients*		
3-month GOS-E[a]	4	1	4	0	U = 485; Z = −1.4; $p = 0.17$	Mann-Whitney U test
	5	6	5	3		
	6	3	6	10		
	7	13	7	8		
	8	18	8	8		
6-month outcome measures						
6-month GOS-E[b]	4	1	4	0	U = 459; z = −0.67; $p = 0.52$	Mann-Whitney U test
	5	4	5	3		
	6	7	6	7		
	7	13	7	9		
	8	14	8	7		
RPQ-3[b] Median (25%, 75%)	2.0 [0.0,4.0]		1.5 [0.0,4.3]		U = 467; z = −0.55; $p = 0.59$	Mann-Whitney U test
RPQ-13[b] Median (25%, 75%)	7.0 [4.0,16.0]		14.0 [3.3,21.0]		U = 441; z = −0.89; $p = 0.38$	
CVLT-II scaled score[c]	54 ± 11		57 ± 9		$t(55) = 0.91$; $p = 0.37$	Two-tailed *t*-test
WAIS IV PSI[d] percentile	58% ± 28%		62% ± 27%		$t(57) = 0.45$; $p = 0.65$	
TMT A[e]						
• Time (sec)	31 ± 13		30 ± 9		$t(59) = -0.37$; $p = 0.71$	Two-tailed *t*-test
• Time (z-score)	0.68 ± 1.45		0.50 ± 1.29		$t(59) = -0.51$; $p = 0.62$	
• TMT A >2 SDs above mean	Yes	7	Yes	3	U = 417; z = −0.88; $p = 0.38$	Mann-Whitney U test
	No	28	No	23		
TMT B[e]						
• Time (sec)	65 ± 27		69 ± 27		$t(59) = 0.51$; $p = 0.61$	Two-tailed *t*-test
• Time (z-score)	0.93 ± 1.75		1.09 ± 1.94		$t(59) = 0.34$; $p = 0.74$	
• TMT B >2 SDs above mean	Yes	8	Yes	8	U = 419; z = −0.69; $p = 0.56$	Mann-Whitney U test
	No	27	No	18		

[a]Three CT/MRI-negative mTBI and 3 CT/MRI-positive mTBI patients did not complete 3-month GOS-E evaluation.
[b]Five CT/MRI-negative mTBI and 6 CT/MRI-positive mTBI patients did not complete 6-month GOS-E, RPQ-3, or RPQ-13.
[c]Eleven CT/MRI-negative mTBI and 8 CT/MRI-positive mTBI patients did not complete 6-month CVLT-II.
[d]Ten CT/MRI-negative mTBI and 7 CT/MRI-positive mTBI patients did not complete 6-month WAIS IV.
[e]Nine CT/MRI-negative mTBI and 6 CT/MRI-positive mTBI patients did not complete 6-month TMT A or TMT B.

CT, computed tomography; MRI, magnetic resonance imaging; mTBI, mild traumatic brain injury; GOS-E, Glasgow Outcome Scale – Extended; CVLT-II, California Verbal Learning Test–Second edition; RPQ, Rivermead Postconcussion Symptoms Questionnaire; TMT, Trail Making Test; SD, standard deviation; WAIS IV PSI, Wechsler Adult Intelligence Scale–Fourth edition, Processing Speed Index.

Table 5. Spearman's Correlation Coefficients (ρ) Between Outcome Measures[a] and Demographic, Socioeconomic, Clinical, and Imaging Predictors[b] in 76 mTBI Patients

	Demographic, clinical, socioeconomic				*Day-of-injury head CT*					*Early brain MRI (11.2 ± 3.3 days postinjury)*		
	Age	*Education (years)*	*Neuropsychiatric history*	*History of alcohol or drug problem*	*Nondepressed calvarial or skull base fracture*	*EDH*	*SDH*	*SAH*	*Any CT contusion*	*Any MRI contusion*	*Any MRI T2* evidence of hemorrhagic axonal injury[b]*	*Any DTI axonal injury (≥1 ROI with FA > 2.2 SDs below control-group mean)*
3-month GOS-E ($N=70$)	−0.30* $p=0.013$	0.27* $p=0.02$	−0.27* $p=0.03$ (18 pos.)	−0.12 $p=0.34$ (34 pos.)	−0.12 $p=0.33$ (12 pos.)	−0.08 $p=0.54$ (3 pos.)	−0.23 $p=0.06$ (9 pos.)	−0.28* $p=0.02$ (6 pos.)	−0.22 $p=0.07$ (5 pos.)	−0.36† $p=0.003$ (11 pos.)	−0.12 $p=0.34$ (24 pos.)	−0.34† $p=0.004$ (23 pos.)
6-month GOS-E ($N=65$)	−0.18 $p=0.16$	0.31* $p=0.011$	−0.30* $p=0.02$ (17 pos.)	−0.18 $p=0.15$ (31 pos.)	−0.13 $p=0.32$ (10 pos.)	0.01 $p=0.97$ (2 pos.)	−0.17 $p=0.18$ (7 pos.)	−0.20 $p=0.11$ (5 pos.)	−0.19 $p=0.14$ (4 pos.)	−0.19 $p=0.12$ (9 pos.)	−0.03 $p=0.84$ (22 pos.)	−0.25* $p=0.04$ (20 pos.)
Abnormal TMT B (>2 SDs above age-adjusted mean) at 6 months ($N=61$)	0.11 $p=0.42$	−0.18 $p=0.17$	−0.02 $p=0.90$ (16 pos.)	0.01 $p=0.94$ (30 pos.)	−0.14 $p=0.27$ (9 pos.)	−0.11 $p=0.40$ (2 pos.)	0.02 $p=0.88$ (7 pos.)	0.09 $p=0.47$ (5 pos.)	−0.16 $p=0.22$ (4 pos.)	0.07 $p=0.61$ (9 pos.)	0.17 $p=0.18$ (22 pos.)	0.32* $p=0.011$ (19 pos.)
6-month RPQ-3 ($N=65$)	0.23 $p=0.07$	−0.23 $p=0.06$	0.36† $p=0.003$ (17 pos.)	0.25* $p=0.045$ (31 pos.)	−0.12 $p=0.32$ (10 pos.)	−0.21 $p=0.09$ (2 pos.)	0.11 $p=0.37$ (7 pos.)	0.01 $p=0.93$ (5 pos.)	0.07 $p=0.56$ (4 pos.)	0.03 $p=0.84$ (9 pos.)	−0.10 $p=0.45$ (22 pos.)	0.18 $p=0.14$ (20 pos.)
6-month RPQ-13 ($N=65$)	0.26* $p=0.04$	−0.28* $p=0.02$	0.31* $p=0.013$ (17 pos.)	0.16 $p=0.20$ (31 pos.)	0.02 $p=0.85$ (10 pos.)	−0.07 $p=0.60$ (2 pos.)	0.19 $p=0.14$ (7 pos.)	0.16 $p=0.21$ (5 pos.)	0.21 $p=0.10$ (4 pos.)	0.12 $p=0.34$ (9 pos.)	0.02 $p=0.85$ (22 pos.)	0.29* $p=0.02$ (20 pos.)

[a]No statistically significant correlation was found between any imaging, demographic, socioeconomic, or clinical predictor and worse performance on 6-month TMT A (either z-score or dichotomized score), TMT B (z-score), CVLT-II (scaled score or dichotomized score), or WAIS-IV PSI (scaled score or dichotomized score), except for correlation of CVLT-II scaled score with years of education ($\rho=0.27$; $p=0.04$) and correlation of age with TMT A z- score ($\rho=-0.33$; $p=0.0097$). Thus, for brevity, these outcome measures are omitted from Table 5.

[b]No statistically significant correlation was found between gender, unemployment, GCS at emergency department arrival, PTA, PTA duration, LOC, or history of previous TBI (with LOC not exceeding 5 min) and any outcome variable. Thus, for brevity, these predictors are omitted from Table 5. There was a trend toward significant correlation between 6-month GOS-E and unemployed status ($\rho=-0.24$; $p=0.056$).

*$p<0.05$ (light-gray boxes); †$p<0.01$ (dark-gray boxes).

CT, computed tomography; MRI, magnetic resonance imaging; EDH,epidural hematoma; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; DTI, diffusion tensor imaging; ROI, region of interest; SD, standard deviation; FA, fractional anisotropy; GOS-E, Glasgow Outcome Scale-Extended; TMT, Trail Making Test; RPQ, Rivermead Postconcussion Questionnaire; CVLT, California Verbal Learning Test; WAIS, Wechsler Adult Intelligence Scale; pos., positive.

TABLE 6A. MULTIVARIABLE ORDINAL LOGISTIC REGRESSION OF 3- AND 6-MONTH GOS-E VERSUS STATISTICALLY SIGNIFICANT CLINICAL, DEMOGRAPHIC, SOCIOECONOMIC, CT, AND MRI PREDICTORS FROM TABLE 5

Outcome variable	*Predictor*	*Predictor values*	*Univariable odds ratio per unit decrease in GOS-E (95% CI),* p *value*	*Multivariable odds ratio of predictor per unit decrease in GOS-E (95% CI),* p *value*	*Multivariable model significance*	*Cox and Snell pseudo-R^2*	*Nagelkerke pseudo-R^2*
3-month GOS-E ($N=70$)	Age	32.4 ± 10.8 years	1.07 per year (1.03, 1.1); $p=0.002^{\dagger}$	1.07 per year (1.03, 1.1); $p=0.002^{\dagger}$	$p=0.00002^{\S}$	34.5%§	36.9%§
	Education	14.5 ± 2.5 years	0.79 per year (0.66, 0.94); $p=0.0101$*	0.79 per year (0.65, 0.96); $p=0.02$*			
	Neuropsychiatric history	Yes (18) No (52)	3.3 (1.2, 8.8); $p=0.02$*	1.9 (0.65, 5.3); $p=0.25$			
	CT subarachnoid hemorrhage	Yes (6) No (64)	$p=0.053$	Excluded because of collinearity (see text)			
	MRI contusion present	Yes (11) No (59)	4.9 (1.5, 16.4); $p=0.0098^{\dagger}$	3.1 (0.87, 11.0); $p=0.08$			
	DTI axonal injury (≥ 1 ROI with FA > 2.2 SD below control-group mean)	Yes (23) No (47)	3.9 (1.5, 10.0); $p=0.005^{\dagger}$	2.6 (0.94, 7.0); $p=0.07$			
6-month GOS-E ($N=65$)	Education	14.8 ± 2.5 years	0.82 (0.68, 0.98); $p=0.03$*	0.90 per year (0.74, 1.08); $p=0.26$	$p=0.013$*	15.3%*	16.3%*
	Neuropsychiatric history	Yes (17) No (48)	3.7 (1.3, 10.5); $p=0.014$*	2.7 (0.92, 7.9) $p=0$ .07			
	Any DTI axonal injury (≥ 1 ROI with FA > 2.2 SD below control-group mean)	Yes (20) No (45)	2.7 (1.01, 7.1); $p=0.048$*	2.5 (0.83, 6.1) $p=0.11$			

Table 6b. Multivariable Linear Regression of 6-Month RPQ-13 Versus Statistically Significant Clinical, Demographic, Socioeconomic, CT, and MRI Predictors From Table 5

Outcome variable	*Predictor*	*Predictor values*	*Univariable standardized coefficient β,* p *value*	*Multivariable standardized coefficient β,* p *value*	F *(degrees of freedom)*	*Overall model significance*	*Adjusted* R^2
6-month RPQ-13 ($N=65$)	Age	32.0 ± 10.8 years	0.32; $p=0.009^{\dagger}$	0.26; $p=0.02$*	$F(4,60)=6.0^{\ddagger}$	$p=0.0004^{\ddagger}$	23.7%‡
	Education	14.8 ± 2.5 years	−0.29; $p=0.02$*	−0.20; $p=0.10$			
	Neuropsychiatric history	Yes (17) No (48)	0.36; $p=0.003^{\dagger}$	0.22; $p=0.07$			
	Any DTI axonal injury (≥1 ROI with FA > 2.2 SDs below control-group mean)	Yes (20) No (45)	0.31; $p=0.012$*	0.21; $p=0.07$			

Table 6c. Univariable Binary Logistic Regression of 6-Month TMT B Versus Statistically Significant Clinical, Demographic, Socioeconomic, CT, and MRI Predictors From Table 5

Outcome variable	*Predictor*	*Predictor values*	*Univariable odds ratio (95% CI),* p *value*	*Multivariable odds ratio (95% CI),* p *value*	*Multivariable model significance*	*Cox and Snell pseudo-*R^2	*Nagelkerke pseudo-*R^2
6-month TMT B > 2 SDs above age-adjusted mean ($N=61$)	Any DTI axonal injury (≥1 ROI with FA > 2.2 SDs below control-group mean)	Yes (19) No (42)	4.5 (1.3, 15.1); $p=0.014$*	4.5 (1.3, 15.1); $p=0.014$*	$p=0.015$*	9.5%*	13.9%*

CT, computed tomography; MRI, magnetic resonance imaging; GOS-E,Glasgow Outcome Scale-Extended; CI, confidence interval; DTI, diffusion tensor imaging; ROI, region of interest; FA, fractional anisotropy; SD, standard deviation; RPQ, Rivermead Postconcussion Questionnaire; TMT B, Trail Making Test B.

*$p \le 0.05$ (light-gray box) †$p \le 0.01$ (medium-gray box) ‡$p \le 0.001$ (dark-gray box) §$p \le 0.0001$ (dark-gray box).

(both age-adjusted scaled score and dichotomized score), were not significantly correlated with any imaging, clinical, demographic, or socioeconomic predictor (with the exception of modest correlations between CVLT-II scaled score and years of education and between age and TMT A z-score), and these outcome measures were thus also omitted from Table 5, for brevity.

Table 5 shows that among demographic, clinical, and socioeconomic predictors, previous history of neuropsychiatric disorder was the most consistent predictor of outcome, demonstrating statistically significant correlations with 3-month GOS-E ($\rho = -0.27$; $p = 0.03$), 6-month GOS-E ($\rho = -0.30$; $p = 0.02$), 6-month RPQ-3 ($\rho = 0.36$; $p = 0.003$), and 6-month RPQ-13 ($\rho = 0.31$; $p = 0.013$).

Among the imaging predictors, DTI evidence of one or more ROIs with abnormally reduced FA (>2.2 SDs below control-group mean) was the most consistent predictor of outcome, demonstrating statistically significant correlations with 3-month GOS-E ($\rho = -0.34$; $p = 0.004$), 6-month GOS-E ($\rho = -0.25$; $p = 0.04$), abnormal 6-month TMT B ($\rho = 0.32$; $p = 0.011$), and 6-month RPQ-13 ($\rho = 0.29$; $p = 0.02$). Among other imaging predictors, MRI evidence of contusion was significantly correlated with 3-month GOS-E ($\rho = -0.36$; $p = 0.003$), as was CT evidence of SAH, though more weakly ($\rho = -0.28$; $p = 0.02$).

Regression of 3- and 6-month outcome measures on demographic, clinical, and imaging predictors

Based on the results of Spearman's correlation analysis (Table 5), we constructed regression models of each of five outcome measures: 3-month GOS-E; 6-month GOS-E; 6-month TMT B (dichotomized score); 6-month RPQ-3; and 6-month RPQ-13. The predictive (independent) variables in the model for each outcome measure were limited to only those predictors that had demonstrated a statistically significant Spearman's correlation with that outcome measure in Table 5. This resulted in a multivariable regression model for four outcome measures (3- and 6-month GOS-E, 6-month RPQ-3, and 6-month RPQ-13) and a univariable regression model for one outcome measure (6-month TMT B dichotomized score). No regression model was constructed for any outcome measure that lacked a statistically significant Spearman's correlation with at least one predictor.

For the 3-month GOS-E, age, number of years of education, neuropsychiatric history, MRI evidence for contusion, and DTI evidence of one or more abnormal ROIs with FA more than 2.2 SDs below the control-group mean demonstrated statistically significant univariable odds ratios (ORs; Table 6A), compatible with the Spearman's correlation results from Table 5. The multivariable model for 3-month GOS-E, including all of these predictors, was also significant (pseudo-R^2 of 34.5–36.9%; $p = 0.00002$; Table 6A). Although CT evidence of SAH demonstrated a nearly statistically significant univariable OR ($p = 0.053$), it was excluded from the multivariable model because of collinearity with MRI evidence of contusion. In particular, unstable ORs and a variance inflation factor >2 were observed for CT evidence of SAH and MRI evidence of contusion when both were simultaneously included in the multivariable model.

For the 6-month GOS-E, years of education, neuropsychiatric history, and DTI evidence of one or more abnormal ROIs with FA more than 2.2 SDs below the control-group mean demonstrated statistically significant univariable ORs (Table 6A), compatible with Spearman's correlation results from Table 5. The multivariable model for 6-month GOS-E, including all of these predictors, was also significant (pseudo-R^2 of 15.3–16.3%; $p = 0.013$; Table 6A).

For 6-month RPQ-13, age, years of education, neuropsychiatric history, and DTI evidence of one or more abnormal ROIs with FA more than 2.2 SDs below the control group mean demonstrated statistically significant univariable ORs, consistent with Spearman's correlation results from Table 5. The multivariable linear regression model for 6-month RPQ-13, including all of these predictors was also significant (adjusted R^2 of 23.7%; $p = 0.0004$; Table 6B).

Because the 6-month TMT B was significantly correlated with only one predictor (Table 5), a univariable binary logistic regression model was constructed for this outcome measure. DTI evidence of one or more ROIs with abnormally reduced FA demonstrated a statistically significant univariable OR of 4.5 ($p = 0.014$; Table 6C).

For 6-month RPQ-3, only neuropsychiatric history and history of drug or alcohol abuse demonstrated statistically significant univariable ORs. The multivariable ordinal logistic regression model for 6-month RPQ-3, including both of these predictors, was also statistically significant (pseudo-R^2 of 9.5–13.9%; $p = 0.015$).

Analysis of subset of patients without pre-existing neuropsychiatric or substance abuse history

Most previous studies of DTI in mTBI have excluded patients with history of neuropsychiatric disease or substance abuse on the grounds that DTI results could be influenced by one or both of these factors. We performed whole-brain voxel-wise nonparametric statistical comparison of FA in CT/MRI-negative patients with a positive history of neuropsychiatric disease or substance abuse ($n = 24$), compared to those without ($n = 20$). Many areas of reduced FA at $p < 0.25$ (though not at $p < 0.05$) were found. Therefore, to address the possibility that a previous history of substance abuse and/or neuropsychiatric disease could have influenced our results, we separately analyzed the subset of mTBI patients without such history. Supplementary Tables S2 and S3 (see online supplementary material at http://www.liebertpub.com) summarize demographic, socioeconomic, and clinical characteristics, and 3- and 6-month outcome measures, for this subset of 37 mTBI patients without history of substance abuse or neuropsychiatric disease.

Figure 2A is analogous to Figure 1A, but compares only mTBI patients without history of neuropsychiatric disorder or substance abuse ($n = 37$) to control subjects ($n = 50$). Unlike Figure 1A, no significant group differences in FA (Fig. 2A), MD, RD, or AD were found.

Analogous to Figure 1B, Figure 2B compares CT/MRI-positive mTBI patients without neuropsychiatric or substance abuse history ($n = 17$) to controls ($n = 50$). There are extensive areas of reduced FA in the CT/MRI-positive mTBI patients, despite the expected loss of statistical power for comparison of this small subgroup of only 17 CT/MRI-positive mTBI patients to controls. No region of increased FA, or of increased or reduced MD, AD, or RD, was observed in CT/MRI-positive mTBI patients, relative to controls, at $p < 0.05$.

Finally, analogous to results in Figure 1C, no significant group differences in FA (Fig. 2C), MD, RD, or AD were found in CT/MRI-negative patients without neuropsychiatric or substance abuse history ($n = 20$), compared to controls ($n = 50$), at $p < 0.05$.

Table 7 shows that all 17 of 17 (100.0%) CT/MRI-positive mTBI patients without neuropsychiatric or substance abuse history had abnormal conventional MRI, but only 5 of 17 (24%) had abnormal head CT. One patient with a nondepressed anterior skull base fracture had no CT or MRI evidence of traumatic brain lesion or intracranial hemorrhage and was classified as CT/MRI-negative mTBI (analogous to the classification of isolated nondepressed skull fracture as uncomplicated mTBI in previous literature[38]). On conventional MRI

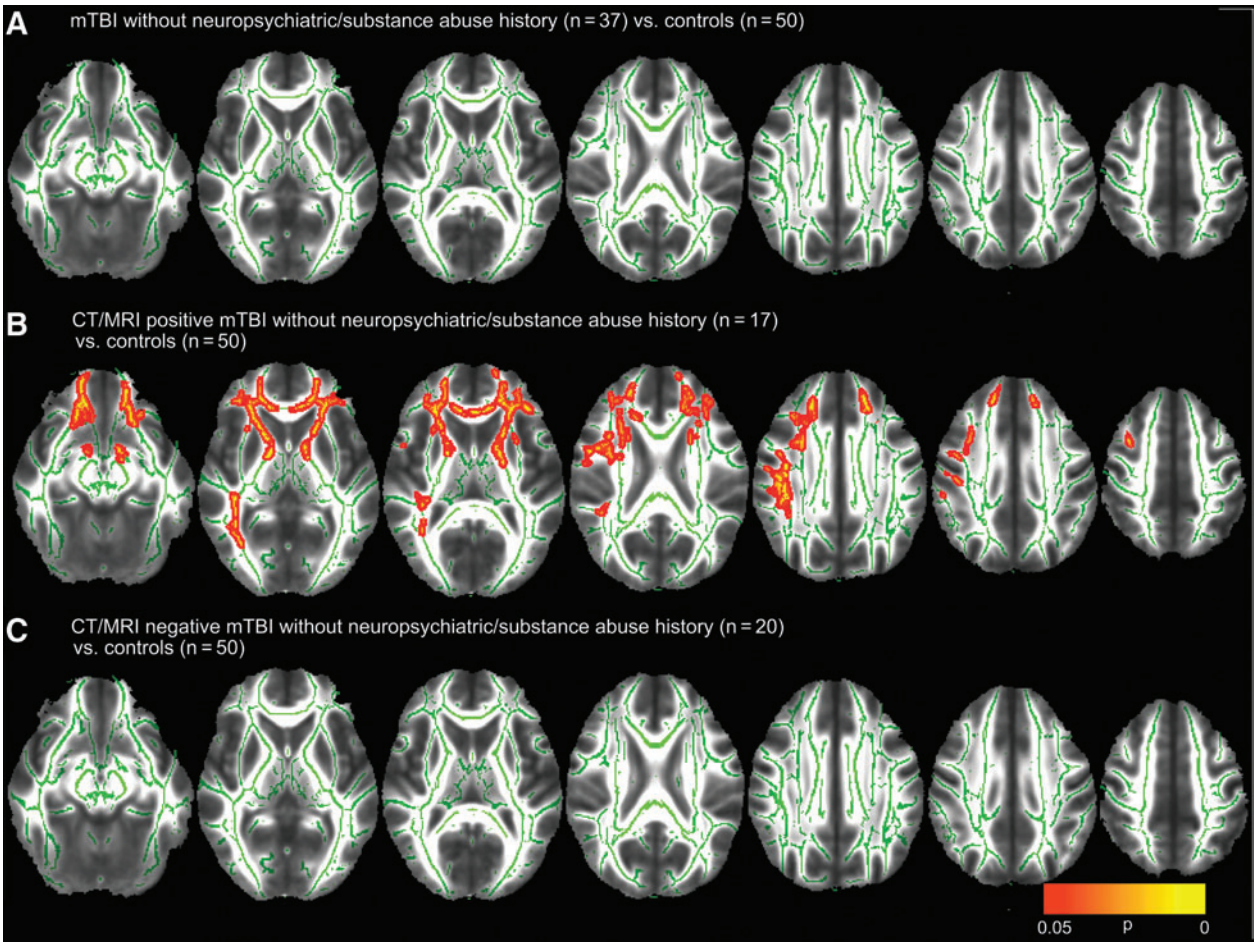

FIG. 2. Voxel-wise nonparametric statistical comparison between mild traumatic brain injury (mTBI) patients without previous history of substance abuse or other neuropsychiatric disorder and controls, with corrections for multiple voxel-wise comparisons using threshold-free cluster enhancement. This analysis was used to compare (**A**) 37 mTBI patients without pre-existing substance abuse or neuropsychiatric history to 50 controls, (**B**) the subgroup of 17 computed tomography/magnetic resonance imaging (CT/MRI)-positive mTBI patients to the 50 controls, and (**C**) the subgroup of 20 CT/MRI-negative patients to the 50 controls. Voxel clusters with statistically significant differences in fractional anisotropy (FA) between mTBI and control groups at $p < 0.05$ are shown in red/orange/yellow, with yellow denoting greater statistical significance. (B) shows that CT/MRI-positive mTBI patients without substance abuse or neuropsychiatric history demonstrated significantly lower FA in the anterior and posterior limbs of the internal capsules, external capsules, uncinate fasciculi, genu of the corpus callosum, and anterior corona radiata bilaterally. In contrast, (C) shows that this method demonstrated no evidence for white matter injury in CT/MRI-negative mTBI. Color image is available online at www.liebertpub.com/neu

sequences, most CT/MRI-positive mTBI patients (11 of 17; 64.7%) demonstrated isolated foci of hemorrhagic axonal injury without brain contusion; 4 of 17 (23.5%) demonstrated both hemorrhagic axonal injury and brain contusion; 1 of 17 (5.9%) demonstrated brain contusions and EDH; and 1 of 17 (5.9%) had isolated SDH.

Tables 7 and 8 also show results of ROI analysis of the 17 CT/MRI-positive and 20 CT/MRI-negative mTBI patients without a history of neuropsychiatric or substance abuse. Table 7 shows lesions with abnormally low FA (FA more than 2.2 SDs below the control-group mean) in individual patients. Table 8 shows that such lesions were observed in ≥ 1 ROIs for 9 of 17 CT/MRI-positive mTBI (52.9%), 2 of 20 CT/MRI-negative mTBI (10.0%), and 5 of 50 (10.0%) control subjects. Fisher's exact test showed a highly significant difference between the proportions of CT/MRI-positive mTBI patients (52.9%) and control subjects (10.0%) with ≥ 1 abnormal ROIs ($p = 0.0006$). There was also a highly significant difference between the proportions of CT/MRI-positive mTBI patients (52.9%) and CT/MRI-negative mTBI patients (10.0%) with ≥ 1 abnormal ROIs ($p = 0.0097$). However, there was no difference in the proportions of CT/MRI-negative mTBI patients (10.0%) and controls (10.0%) with ≥ 1 abnormal ROIs ($p = 1.0$). Finally, there was no significant difference among CT/MRI-positive mTBI, CT/MRI-negative mTBI, and control subject groups in terms of the proportion of subjects with ≥ 1 ROI with abnormally high FA ($p = 0.75$).

Table 9 is analogous to Table 5 and shows the pairwise Spearman's correlations between 3- and 6-month outcome measures and demographic, socioeconomic, clinical, CT, and MRI predictors in patients without a history of neuropsychiatric or substance abuse. Except for an expected correlation[52] of years of education with TMT B z-score ($\rho = -0.50$; $p = 0.007$), and correlation of TMT A z-score with age ($\rho = -0.39$; $p = 0.04$) and with PTA duration ($\rho = 0.48$; $p = 0.014$), no demographic, socioeconomic, or clinical variable (age, gender, employment status, GCS, PTA, PTA duration, LOC, or history of earlier TBI) was otherwise significantly correlated at $p < 0.05$ with worse performance on any outcome measure; all demographic,

TABLE 7. CT, CONVENTIONAL MRI AND DTI FINDINGS IN CT/MRI-POSITIVE AND CT/MRI-NEGATIVE mTBI PATIENTS WITHOUT PRE-EXISTING SUBSTANCE ABUSE OR NEUROPSYCHIATRIC HISTORY

Patient	*ACR Left*	*ACR Right*	*ALIC Left*	*ALIC Right*	*EC Left*	*EC Right*	*SLF Left*	*SLF Right*	*SS Left*	*SS Right*	*CGH Left*	*CGH Right*	*CT findings*	*Conventional MRI findings*
CT/MRI-positive mTBI														
1	x		x	x									Normal	2 microhemorrhages (R posterior limb of internal capsule)
2					x	x							Normal	1 microhemorrhage (posterior L temp WM)
3											x		Normal	1.3 cm R medial orbital gyr contusion; 2 microhemorrhages (R periventricular).
4								x					SDH; SAH; nondisplaced skull fracture	L sup, mid, inf temp gyr, L fr opercular contusions; 2 microhemorrhages (L & R CGH).
5								x					Normal	2 microhemorrhages (L CGH, L sup fr gyr)
6			x										Normal	3 microhemorrhages (R genu, L sup fr gyr)
7									x				3 mm L SDH; L temp SAH	L mid and inf fr gyr, L sup and mid-temp gyr contusions; 2 microhemorrhages (L post temp, R postcentral gyr).
8		x											Small B SDH; B fr contusion; SAH	R frontal, B occ contusions; 3 microhemorrhages (B ant temp & R occ WM)
9	x												EDH; SAH; L temp contusion	R ant temp, L inf fr, R gyr rectus, R medial orbital gyr contusions; EDH
10													Normal	2 microhemorrhages (R CGH, L post temp WM)
11													Normal	2 microhemorrhages (L precentral gyr, L sup fr gyr)
12													Normal	4 microhemorrhages (L sup fr gyr, R fr operculum)
13													Normal	2 microhemorrhages (L ant and post temp WM)
14													Normal	1 microhemorrhage (R ant temp)
15													Normal	2 microhemorrhages (L sup parietal lobule)
16													Normal	2 microhemorrhages (L and R ant temp WM)
17													Small L SDH	Small L SDH

(*continued*)

Table 7. (Continued)

Patient	*ACR Left*	*ACR Right*	*ALIC Left*	*ALIC Right*	*EC Left*	*EC Right*	*SLF Left*	*SLF Right*	*SS Left*	*SS Right*	*CGH Left*	*CGH Right*	*CT findings*	*Conventional MRI findings*
CT/MRI-negative mTBI														
1	**x**												Normal	Normal
2										**x**			Normal	Normal
3													Nondepressed anterior skull base fracture	Normal
4													Normal	Normal
5													Normal	Normal
6													Normal	Normal
7													Normal	Normal
8													Normal	Normal
9													Normal	Normal
10													Normal	Normal
11													Normal	Normal
12													Normal	Normal
13													Normal	Normal
14													Normal	Normal
15													Normal	Normal
16													Normal	Normal
17													Normal	Normal
18													Normal	Normal
19													Normal	Normal
20													Normal	Normal
Mean FA (controls)	0.56	0.55	0.65	0.64	0.53	0.51	0.57	0.55	0.64	0.63	0.67	0.64		
Mean FA −2.2 SDs	0.50	0.49	0.60	0.59	0.48	0.46	0.50	0.50	0.58	0.57	0.57	0.53		

MRI, magnetic resonance imaging; DTI, diffusion tensor imaging; CT, computed tomography; mTBI, mild traumatic brain injury; ACR, anterior corona radiata; ALIC, anterior limb internal capsule; EC, external capsule; SLF, superior longitudinal fasciculus, SS, sagittal striatum; CGH, cingulum (parahippocampal gyrus); SDH, subdural hematoma; SAH, subarachnoid hemorrhage; L, left; R, right; B, bilateral; EDH, epidural hematoma; WM, white matter; sup, superior; mid, middle; inf, inferior; ant, anterior; post, posterior; fr, frontal; temp, temporal; occ, occipital; gyr, gyrus; SD, standard deviation; ROI, region of interest.

Extraaxial collection only
Microhemorrhage(s)
Contusion(s)
Contusion(s) + microhemorrhage(s)
Color table is available online at www.liebertpub.com/neu

Table 8. DTI Region-of-Interest (ROI) Analysis: Group Differences in Presence of One or More Abnormal ROIs among CT/MRI-Negative mTBI and CT/MRI-Positive mTBI without Neuropsychiatric or Substance Abuse History and Control Subjects

	CT/MRI-negative mTBI (20 subjects)	*CT/MRI-positive mTBI (17 subjects)*	*Controls (50 subjects)*
	Number of subjects (Proportion of subjects)	*Number of subjects (Proportion of subjects)*	*Number of subjects (proportion of subjects)*
One or more ROIs with FA more than 2.2 SDs below control-group mean	2 (10.0%)[a]	9 (52.9%)[b]	5 (10.0%)[a]
One or more ROIs with FA more than 2.2 SD above control-group mean	3 (15.0%)[c]	1 (5.9%)[c]	5 (10.0%)[c]

[a,b,c]Each superscript denotes a subset of participants whose column proportions do not differ significantly from one another, by Fisher's exact test with $p < 0.05$. Row 1: There was a significant difference between the proportions of CT/MRI-positive (52.9%) and CT/MRI-negative mTBI patients (10.0%) with one or more ROIs with FA more than 2.2 SDs below the control group mean ($p = 0.0097$). There was also a highly significant difference between CT/MRI-positive mTBI patients (52.9%) and controls (10.0%; $p = 0.0006$). However, there was no difference between CT/MRI-negative mTBI patients (10.0%) and controls (10.0%; $p = 1.0$). Row 2: There was no significant difference among the proportions of CT/MRI-negative mTBI (15.0%), CT/MRI-positive mTBI (5.9%), and control subjects (10.0%) with one or more ROIs with FA more than 2.2 SDs above the control group mean ($p = 0.75$).

CT, computed tomography; MRI, magnetic resonance imaging; DTI, diffusion tensor imaging; ROI, region of interest; mTBI, mild traumatic brain injury; FA, fractional anisotropy; SD, standard deviation.

socioeconomic, and clinical variables were thus excluded from Table 9 for brevity. Similarly, 6-month TMT A (both age-adjusted z-score and the dichotomized score), TMT B (z-score), CVLT-II (both age-adjusted scaled score and dichotomized score), and WAIS-IV PSI (both age-adjusted scaled score and dichotomized score) were also omitted from Table 9 because they demonstrated no other significant correlation with any other imaging, clinical, demographic, or socioeconomic predictor at $p < 0.05$.

Table 9 shows that among the imaging predictors, no CT feature (CT evidence of nondepressed skull fracture, EDH, SDH, SAH, or contusion) was significantly correlated with any outcome measure at $p < 0.05$. In contrast, several MRI features, including MRI evidence of contusion, MRI evidence of hemorrhagic axonal injury, and presence of abnormally reduced FA in at least one ROI, demonstrated statistically significant correlations with several outcome measures (3- and 6-month GOS-E, abnormal 6-month TMT B, and the 6-month RPQ-13).

Discussion

In the current study, white matter FA was significantly reduced in CT/MRI-positive, but not in CT/MRI-negative, mTBI patients, compared to healthy control subjects, on a group level. In addition, regions of reduced FA in individual mTBI patients were modest, but statistically significant, predictors of unfavorable 3- and 6-month outcome. These results held true for both the inclusive sample of 76 mTBI patients as well as the subset of 37 mTBI patients with no history of previous substance abuse or other neuropsychiatric disorder.

Previous studies have reported evidence of white matter injury on DTI in the acute-to-subacute time period after mTBI.[15–18,20,23–25,27–31,34–36] In essentially all of these studies, patients with history of substance abuse or other neuropsychiatric disorders were excluded. In addition, in nearly all of these studies, the mTBI study population included a mixed group of both CT/MRI-positive and -negative mTBI, based on presence of intracranial abnormalities on CT alone, CT and 1.5T MRI, or CT and 3T MRI. Miles and colleagues[31] found, using an ROI approach, reduced average FA and increased average MD within six ROIs in a group-wise comparison of 17 mTBI patients, studied within 10 days of injury at 1.5T MRI and with no evidence of microhemorrhages, to 29 age- and gender-matched controls. In contrast, Ling and colleagues[24] found increased FA and decreased RD, within the callosal genu, in a mixture of 28 CT/MRI-negative and -positive mTBI patients who underwent MRI 15.6 ± 4.3 days after injury. Messe and colleagues,[30] using a whole-brain voxelwise approach to study a mixture of CT/MRI-negative and -positive mTBI patients, found higher MD values in poor-outcome patients, compared to good-outcome patients and controls, in the corpus callosum, right anterior thalamic radiations, superior longitudinal fasciculus, and inferior longitudinal and fronto-occipital fasciculi at 7–28 days after injury. Lange and colleagues,[23] using an ROI approach, found no significant difference in FA or MD in the genu, body, or splenium of the corpus callosum in 60 CT/MRI-positive and -negative mTBI patients (on the more severe end of the mTBI spectrum), relative to 34 trauma controls. A smaller number of studies[20,25,27,35] has reported statistically significant group-wise or individual FA differences in the acute-to-subacute time period in strictly CT/MRI-negative mTBI patients versus controls. For example, Lipton and colleagues, using a whole-brain voxelwise approach, found reduced FA in multiple white matter regions at 2–14 days postinjury in 20 CT/MRI-negative mTBI patients, compared to 20 age- and gender-matched controls.[27] McAllister and colleagues[56] found a statistically significant correlation between mean and maximum strain rate (based on measurements from instrumented helmets and finite element biomechanical simulation) and increased FA in the corpus callosum within the first 10 days after concussion in athletes with normal conventional brain MRI.

From the above, it is evident that DTI analysis techniques have varied between more data-driven, whole-brain voxel-wise analyses and hypothesis-driven ROI approaches. In addition, although nearly all studies have employed group-comparison designs, some investigators have chosen to compare mTBI patients to healthy controls (in some cases, matched by age, gender, and/or education), whereas others have compared mTBI subgroups with good versus poor outcome. These earlier studies, most of which are limited by small sample sizes, have also not analyzed DTI results in the context of important clinical, demographic, and socioeconomic factors relevant to TBI outcomes. Finally, there is a persistent and striking inconsistency across different DTI studies, in terms of the reported direction of changes in DTI measures after mTBI.

Whole-brain voxel-wise approaches may have limited sensitivity as a result of the heterogeneity of spatial distribution of white matter

Table 9. Spearman's Correlation Coefficients (ρ) between Outcome Measures and Early Neuroimaging Pathoanatomic Findings in 37 mTBI Patients without Previous history of Substance Abuse or Other Neuropsychiatric Disorder[a]

	Day-of-injury head CT						*Early brain MRI (10.9±3.6 days postinjury)*			
	Nondepressed calvarial or skull base fracture	*EDH*	*SDH*	*SAH*	*Any CT Contusion*	*Any acute traumatic intracranial CT finding*	*Any MRI contusion*	*Any MRI T2* hemorrhagic axonal injury*	*Any DTI axonal injury (≥1 ROI with FA > 2.2 SDs below control-group mean)*	*Any conventional MRI and/or DTI lesion*
3-month GOS-E ($N=32$)	−0.15 $p=0.40$ (4 positive)	−0.05 $p=0.78$ (1 positive)	−0.24 $p=0.19$ (5 positive)	−0.28 $p=0.12$ 3 (positive)	−0.28 $p=0.12$ (5 positive)	−0.24 $p=0.19$ (5 positive)	−0.47 $p=0.006$* (5 positive)	−0.41 $p=0.02$ (12 positive)	−0.50 $p=0.004^{\dagger}$ (10 positive)	−0.37 $p=0.04$* (14 positive)
6-month GOS-E ($N=30$)	−0.06 $p=0.75$ (3 positive)	−0.06 $p=0.76$ (1 positive)	−0.21 $p=0.26$ (3 positive)	−0.08 $p=0.67$ (2 positive)	−0.21 $p=0.26$ (2 positive)	−0.21 $p=0.26$ (3 positive)	−0.22 $p=0.25$ (4 positive)	−0.29 $p=0.12$ (11 positive)	−0.30 $p=0.11$ (7 positive)	−0.39 $p=0.03$* (13 positive)
Abnormal TMT A (>2 SDs above mean) at 6 months ($N=27$)	−0.14 $p=0.50$ $N=2$ (2 positive)	−0.09 $p=0.64$ $N=27$ (1 positive)	−0.17 $p=0.40$ $N=27$ (3 positive)	−0.14 $p=0.50$ $N=27$ (2 positive)	−0.14 $p=0.50$ $N=27$ (2 positive)	−0.17 $p=0.40$ $N=27$ (3 positive)	−0.20 $p=0.32$ (4 positive)	0.01 $p=0.97$ (11 positive)	0.15 $p=0.45$ (7 positive)	0.11 $p=0.57$ (13 positive)
Abnormal TMT B (>2 SDs above mean) at 6 months ($N=27$)	−0.17 $p=0.40$ (2 positive)	−0.12 $p=0.56$ (1 positive)	0.06 $p=0.77$ (3 positive)	0.16 $p=0.44$ (2 positive)	−0.17 $p=0.40$ (2 positive)	0.06 $p=0.77$ (3 positive)	0.23 $p=0.25$ N=27 (4 positive)	0.20 $p=0.32$ $N=27$ (11 positive)	0.42 $p=0.03$* N=27 (7 positive)	0.28 $p=0.16$ $N=27$ (13 positive)
6-month RPQ-3 ($N=30$)	−0.10 $p=0.60$ (3 positive)	−0.21 $p=0.26$ (1 positive)	0.13 $p=0.48$ (3 positive)	−0.02 $p=0.90$ (2 positive)	0.03 $p=0.87$ (2 positive)	0.13 $p=0.48$ (3 positive)	0.27 $p=0.15$ (4 positive)	0.32 $p=0.09$ (11 positive)	0.12 $p=0.54$ (7 positive)	0.23 $p=0.22$ (13 positive)
6-month RPQ-13 ($N=30$)	−0.06 $p=0.74$ (3 positive)	−0.13 $p=0.50$ (1 positive)	0.22 $p=0.25$ (3 positive)	0.04 $p=0.84$ (2 positive)	0.13 $p=0.49$ (2 positive)	0.22 $p=0.25$ (3 positive)	0.22 $p=0.25$ (4 positive)	0.62 $p=0.0003^{\dagger}$ (11 positive)	0.40 $p=0.03$* (7 positive)	0.61 $p=0.0004^{\dagger}$ (13 positive)

[a]The only statistically significant pair-wise correlations between any demographic, clinical or socioeconomic predictor and worse performance on any outcome variable were between years of education and TMT B z-score ($\rho=-0.50$; $p=0.007$), age and TMT A z-score ($\rho=-0.39$; $p=0.04$), and PTA duration and TMT A z-score ($\rho=0.48$; $p=0.014$). Thus, for brevity, all demographic, clinical, and socioeconomic predictors (Supplementary Table 2) (see online supplementary material at http://www.liebertpub.com) are omitted from Table 9. Similarly, 6-month TMT A (both z-score and dichotomized score), TMT B (z-score), CVLT-II (scaled score and dichotomized score), and WAIS-IV PSI (scaled score and dichotomized score) were omitted from Table 9, because they demonstrated no other significant correlation with any imaging, clinical, demographic, or socioeconomic predictor at $p<0.05$. *$p<0.05$ (light-gray boxes); $^{\dagger}p<0.01$ (dark-gray boxes).

CVLT-II, California Verbal Learning Test–Second Edition; EDH,epidural hematoma; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; DTI, diffusion tensor imaging; ROI, region of interest; SD, standard deviation; FA, fractional anisotropy; GOS-E, Glasgow Outcome Scale-Extended; TMT, Trail Making Test; RPQ, Rivermead Postconcussion Questionnaire; CVLT, California Verbal Learning Test; WAIS-IV PSI, Wechsler Adult Intelligence Scale–Fourth Edition, Processing Speed Index.

injury in mTBI; on the other hand, the ROI approach may be limited by failure to interrogate less-common areas of white matter injury. We employed both of these as complementary approaches in the current study and demonstrated that microstructural white matter injury severity does vary, on a group level, according to the presence of more-familiar macroscopic pathoanatomic lesions on CT and conventional MRI. It may not be surprising that the data show that CT/MRI-positive mTBI patients have more extensive white matter injury than CT/MRI-negative mTBI patients. However, such work is relevant because any utility of DTI in outcome prediction would be contingent on demonstration of a differential increase in diagnostic or prognostic accuracy beyond conventional CT and MRI as well as clinical, demographic, and socioeconomic predictors.

In this study of 76 mTBI patients and 50 control subjects, and using current DTI acquisition and postprocessing techniques, CT/MRI-positive mTBI patients demonstrated evidence of white matter injury when employing either whole-brain voxel-wise or ROI approaches. Indeed, we found no evidence for white matter injury, using either the whole-brain voxel-wise or ROI methods, in mTBI patients without lesions on CT or 3T MRI that included high-resolution 3D T1- and T2-weighted sequences as well as T2*-weighted gradient echo sequences. These findings held true in both the inclusive group of 76 mTBI patients, as well as the subset of 37 patients with no previous history of substance abuse or other neuropsychiatric disorders. There are several possible reasons for the discrepancy between our results with a few earlier studies demonstrating statistically significant FA differences on acute-to-subacute 3T DTI between strictly CT/MRI-negative mTBI patients and controls.[20,25,27,35] Technical differences in DTI acquisition or DTI postprocessing techniques could always be an explanation for such differences. The effect size and incidence of white matter injury in CT/MRI-negative mTBI may be too small, or the severity and/or spatial distribution too variable among patients, to show statistically significant group differences based on the number of patients and analysis approach employed in the current study. The injury-to-MRI interval may be a critical factor; it has been postulated that a variety of different biological processes within injured white matter may vary not only according to injury severity, but also at different time intervals after injury, and that FA, in particular, may be abnormally increased within the first week of injury.[16,18,29,35,36] Patients in the current study underwent MRI during the first 3 weeks after injury (11.2 ± 3.3 days), when different biological processes and thus DTI parameters may still have been evolving. Finally, it is possible that our results differ because many cases of CT/MRI-positive mTBI in this study were placed in that group on the basis of very subtle MRI lesions at 3T, such as one or two subtle isolated foci of hemorrhagic axonal injury, and may have been classified as uncomplicated mTBI in other studies. This third explanation has the appeal of being compatible with earlier literature that reports DTI evidence of white matter injury in subjects classified as uncomplicated mTBI based on CT alone.[15,16,18,36] Another main aim of this work was to investigate the utility of DTI parameters as predictors of individual outcome. We thus determined and compared ORs for a variety of demographic, socioeconomic, clinical, and imaging predictors, including DTI parameters. Our data suggest that MRI predictors, particularly MRI evidence of contusion and DTI evidence of one or more ROIs with reduced FA, and clinical and socioeconomic predictors, including education and previous history of neuropsychiatric disorder, surpass most CT features for prediction of most 3- and 6-month outcome measures.

Analysis of the subset of mTBI patients without a previous history of substance abuse and/or neuropsychiatric disease (Fig. 2; Tables 7–9 and Supplementary Tables 2 and 3) (see online supplementary material at http://www.liebertpub.com) is informative, because it addresses the problem of a possible strong confounding influence of these pre-existing conditions owing to their potential relationships with *both* DTI parameters and outcome. In this subset analysis, it was actually necessary to separate CT/MRI-positive from CT/MRI-negative mTBI patients to see any evidence of white matter injury using either the whole-brain voxel-wise or ROI approaches. Specifically, the whole-brain voxel-wise analysis (Fig. 2) and ROI analysis (Tables 7 and 8) both demonstrate differences between CT/MRI-positive and -negative mTBI patients that are even more striking and statistically significant than in the original analysis of the inclusive group of 76 mTBI patients. Table 8 shows a strikingly higher prevalence of abnormal ROIs with reduced FA in CT/MRI-positive patients without previous history of substance abuse or other neuropsychiatric disorders, relative to both the CT/MRI-negative mTBI patients ($p = 0.004$) and the control group ($p = 0.0002$); in contrast, the same prevalence of abnormal ROIs with reduced FA was observed in CT/MRI-negative patients (10.0%) and in the control group (10.0%).

It is noteworthy that both conventional MRI and DTI predictors demonstrated stronger correlation coefficients with 3- and 6-month outcome measures in the *subset* of 37 patients lacking any history of neuropsychiatric disease or substance abuse (Table 9) than in the larger inclusive sample of 76 patients (Table 5), despite the much smaller sample size of the former. We postulate that this is because correlations of pre-existing factors, such as neuropsychiatric disease, with the outcome measures (e.g., in Table 5) may have weakened the apparent influence or relevance of the imaging predictors.

It is also notable that there were generally much stronger correlations of MRI predictors with 3-month GOS-E than with 6-month GOS-E. This is plausible, because the MRI exams in this study were performed within 3 weeks after mTBI. Abnormal MRI features in the initial days after injury, which demonstrated a strong correlation with 3-month GOS-E, may be less relevant at 6 months, after a variable degree of recovery has taken place in different patients. The stronger correlation with the GOS-E at 3 months, compared to 6 months, is unlikely to be attributable solely to general overall improvement in the GOS-E over time: Though many individual patients' scores changed between the two time points, there was negligible change in the overall distribution of GOS-E scores at 3 versus 6 months (Table 4 and Supplementary Table 3) (see online supplementary material at http://www.liebertpub.com).

In this study, we sought to minimize the influence of confounding factors on group differences in DTI parameters between patient and control groups. Thus, we did not follow the approach of presorting patients according to an outcome measure, and thereafter assessing for group differences in DTI results according to good or poor outcome, because there are many potential confounding factors that could affect both DTI measures and outcome. Further, we analyzed, in addition to the original inclusive sample, the subset of patients lacking any significant reported substance abuse or other neuropsychiatric history, because these pre-existing conditions are heterogeneous by nature and thus difficult to control for in group comparisons and could act as confounding variables that could create or exacerbate group differences in DTI measures. Finally, because there was a nonsignificant, but noticeable, difference in number of years of education among CT/MRI-positive mTBI, CT/MRI-negative mTBI, and control groups, we explicitly demonstrated that there were no group differences in DTI measures, using either the DTI or ROI approach, between the most- and least-educated control subjects.

This study has several limitations. Alteration of DTI parameters in TBI has been linked to a variety of possible pathophysiological mechanisms, such as axonal disruption, axonal degeneration, and cytotoxic edema; recent work also suggests that DTI parameters, such as FA and MD, may be correlated with strain and strain rate in mTBI.[56] Nevertheless, despite our attempt, in performing the subset analysis, to minimize or eliminate the influence of confounding factors that could account for both DTI lesions and poorer outcome, we acknowledge that lesions in the DTI ROI analysis are nonspecific and may reflect the patient's pre-existing brain structure, rather than a traumatic lesion.[33] Second, a substantial unexplained variance in outcomes remains, even for our most inclusive models that were based on DTI, conventional neuroimaging, and other predictors (Table 6). Third, because the number of predictors we investigated was large, relative to the number of patients, this study should be regarded as exploratory and in need of confirmation in a larger study population. Finally, even for pathoanatomic findings, such as contusion and SAH, that can be definitively attributed to acute TBI based on their unique imaging appearance, the existence of any direct pathophysiological mechanism that accounts for their correlation with outcome remains uncertain.

In summary, this study provides evidence for the importance of individual pathoanatomic features on MRI, including DTI parameters, for prognosis after mTBI. Specifically, several MRI predictors, including DTI parameters, surpassed CT features for prediction of 3- and 6-month outcome measures. For the subset of patients lacking any significant neuropsychiatric or substance abuse history, MRI predictors, including DTI parameters, surpassed all clinical, demographic, socioeconomic, and CT features for prediction of 3- and 6-month outcome. Our results should be viewed as relevant primarily to mTBI patients who meet ACEP/CDC ED criteria for head CT and who thus generally have more severe injuries than mTBI patients who are not triaged to head CT. Our results support the potential utility of MRI and DTI in the acute/subacute stage of acute mTBI for better classification of injury severity. Effective, practical imaging markers that identify mTBI patients who will have unfavorable outcome are essential for clinical trials to evaluate treatments and for better triage to effective follow-up care.

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Author Disclosure Statement

No competing financial interests exist

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